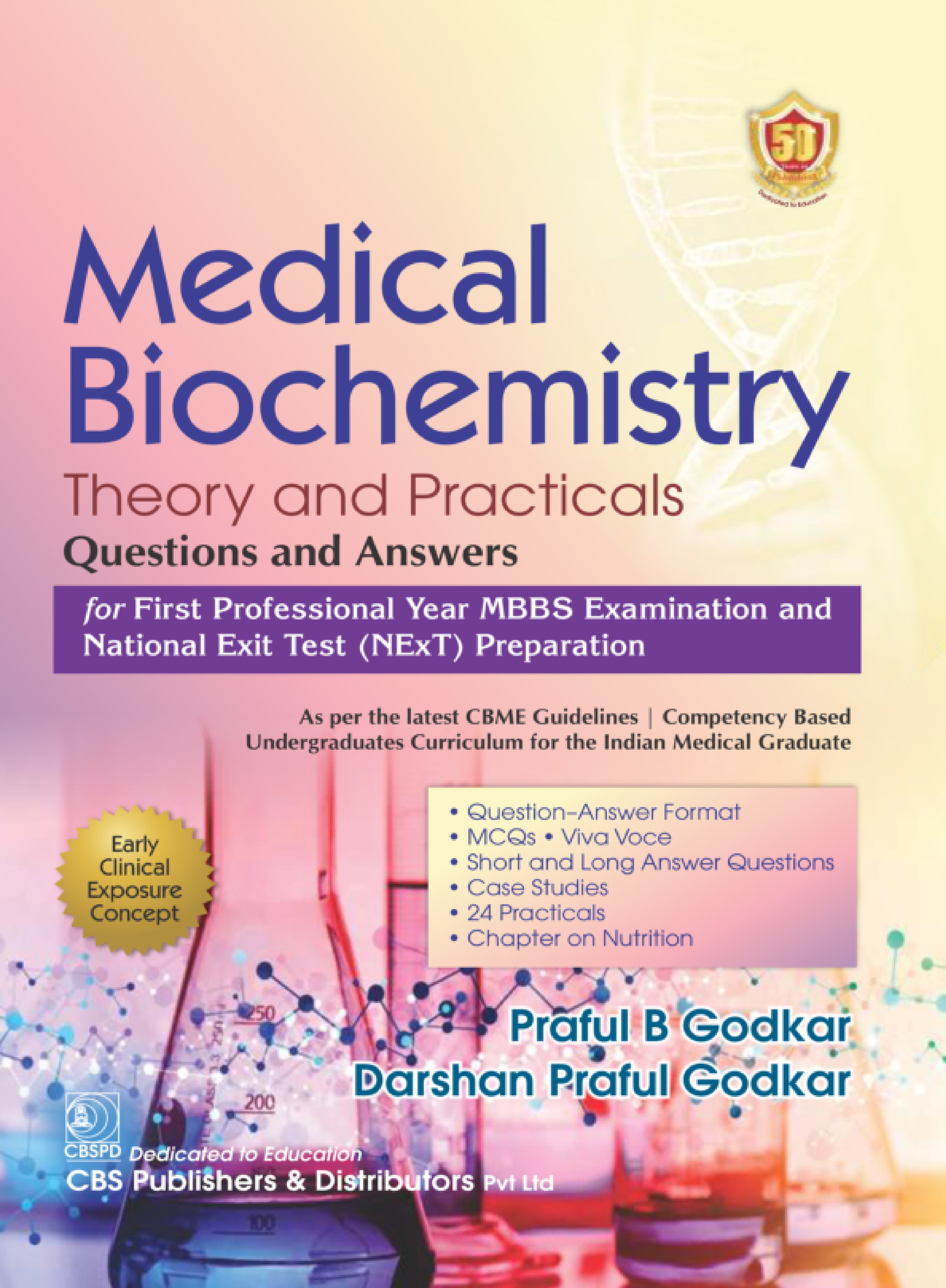

Medical Biochemistry

Theory and Practicals

Questions and Answers

for First Professional Year MBBS Examination and National Exit Test (NExT) Preparation

As per the latest CBME Guidelines | Competency Based Undergraduates Curriculum for the Indian Medical Graduate

Early Clinical Exposure Concept

- Question–Answer Format
- MCQs • Viva Voce
- Short and Long Answer Questions
- Case Studies
- 24 Practicals
- Chapter on Nutrition

Praful B Godkar
Darshan Praful Godkar

CBSPD *Dedicated to Education*
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to

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my grandchildren
Radha (Ninu), Yash and Krisha

who kept me on-line busy during the entire COVID-19:
Pandemic phase of the twenty-first century, and also for providing
me an angle of understanding primary and secondary educational concepts

Introduction

This question-and-answer book on medical biochemistry is based on the complete contents of the new syllabus of the National Medical Commission (2019) and the proposed National Exit Test (NExT).

Types of questions of 1st year MBBS pre-clinical subject: Medical biochemistry is comprised of the following types of questions:

Theory

A. Multiple choice questions (MCQs)
B. Short answer questions:
  Types: Define, give reasons, name two, three, or four types of specific aspects, enumerate factors, briefly describe specific topics, etc.
C. Long answer questions
D. Questions based on "case histories"
E. Questions based on "case description with a biochemistry laboratory report".
F. Minimum marks for passing: 50

Table Viva Voce Questions

Similar to short answer questions

Practical Examination Viva Voce

Questions based on clinical biochemistry practicals,

NExT is comprised of the following two separate examinations:

1. NExT step1: Theory examination

2. NExT step 2: Practical/clinical and viva voce examination.

1. NExT step1: Theory examination

Question type: Multiple-choice questions

A. Examination mode: Computer-based online centralized all-India examination.
B. Subjects of III MBBS/final MBBS part 1 and part 2 and applied aspects of subjects of the first year MBBS and second year MBBS.
C. Minimum marks for passing: 50%

2. NExT step 2: Practical/clinical and viva voce

A. Examination type: Objective structured, clinical case-based, simulated cases, patients aimed at practical/clinical skills, clinical decision-making, communication skills
B. Examination mode: Examination shall be conducted live and conducted by respective state health universities.
C. Minimum marks for passing: 50%

References

1. Syllabus of National Medical Commission (2019)
2. National Exit Test (NExT).

How to Use This Book

This book will be useful for the students for the following purposes:

- To get basic knowledge of biochemistry.
- To get knowledge of medical biochemistry.
- To get knowledge of clinical biochemistry.
- To prepare for theory examination.
- To prepare for the practical examination
- To prepare for the table viva voce examination
- To prepare for the practical viva voce examination
- To prepare for foreign university qualifying examinations such as USMLE, MCAT, OMET, etc.
- To prepare for interviews
- To use as a reference book during medical practice

Mission

At the end of the completion of the first year of learning medical biochemistry, students should be able to:

1. Become competent in the evaluation of laboratory test reports for the diagnosis and management of common health problems of the patients using clinical skills based on history, physical examination and relevant laboratory tests.
2. Become competent to practice preventive, curative, and rehabilitative measures concerning the clinical conditions learned in biochemistry studies.
3. Develop the attitude for continued self-learning and take further interest to pursue research work in any specific area of medicine.
4. Get familiar with the basic factors related to medical biochemistry; which are essential for the implementation of the national health programmes including practical aspects of the following:
   A. Family Welfare and Material and Child Health (MCH)
   B. Prevention and control of communicable and non-communicable diseases.
   C. Immunization
   D. Health education
5. Develop attitudes required for professional life (sense of responsibility, personal integrity and concern for other individuals)

Objectives

At the end of the completion of the medical biochemistry curriculum, the student should be able to demonstrate his/her knowledge and understanding of the following topics:

1. Molecular and functional organization of a cell, sub-cellular components, basic levels of body organization, cellular injury and death.
2. Basic aspects of enzymology, regulation of enzymatic activity and clinical aspects of laboratory tests related to the enzymes.
3. Digestion, absorption, and assimilation of nutrients and clinical significance related to malnutrition.
4. Integration of the various aspects of carbohydrates, proteins, lipids, vitamins, electrolytes, water metabolism, regulatory pathways, disturbed metabolism and related pathophysiology.
5. Biochemical basis of inherited disorders, laboratory tests and their associated clinical significance.
6. Mechanisms involved in the maintenance of body fluid and pH homeostasis, clinical conditions and laboratory tests related to disturbed water and mineral metabolism, laboratory tests and clinical significance.
7. Basic molecular biology, mechanisms of gene expression and regulation, basic molecular pathology, the principles of genetic engineering, gene therapy and their application in medicine.
8. Molecular concepts of body defense mechanisms, laboratory tests and their applications in medicine.
9. Biochemical basis of cancer and carcinogenesis, tumor markers and related clinical significance.
10. Principles of metabolism and detoxication of xenobiotics.
11. Principles of various conventional and specialized laboratory instruments, specimen collection, patient care, and laboratory test methods.
12. Biochemistry laboratory tests, specimen collection, principles, clinical significance and interpretation of laboratory reports.
13. The ability to suggest routine, organ profile and additional laboratory tests to support theoretical concepts and clinical diagnosis.

Developing Skills

At the end of the course, the student should be able to:

1. Make use of conventional techniques and instruments to perform biochemical analysis relevant to clinical screening and diagnosis.
2. Collect specimens, analyze specimens, and interpret laboratory reports.
3. Demonstrate skills in solving clinical problems and decision making

Preface

> *"It is easy to get a thousand prescriptions, but hard to get one single remedy."* — Chinese proverb, which proved 100% correct in the 2019 pandemic phase.

It gives us great pleasure to introduce the first edition of *Medical Biochemistry: Theory and Practicals* in question and answer format for medical students. This was a long-standing request from the medical students during our recent educational sessions. This book includes basic biochemistry and medical biochemistry, theory and practicals, according to the undergraduate competency-based medical education (CBME) curriculum and the latest complete syllabus suggested by National Medical Commission (NMC).

The layout of this book has been organized by keeping in mind the importance of basic biochemistry for the development of fundamental concepts and the enormous impact of medical biochemistry on the understanding and maintenance of health and understanding pathophysiology related to various diseases.

This book will be useful for the students to understand the scientific basis of the life processes at the molecular level and the subject matter will help the students to integrate molecular events with the structure and function of the human body in health and disease.

Our introduction of the CBME curriculum in this book will be useful for the students to integrate medical biochemistry knowledge with other pre-medical subjects such as anatomy, physiology and nutrition and also with paramedical subjects such as general medicine, microbiology and pharmacology and with medical subjects such as ophthalmology, pediatrics, gynecology and obstetrics.

The early clinical exposure (ECE) concept is very important for learning deranged metabolic events and integration of various pre-clinical, para-clinical and clinical subjects. We introduced the ECE concept for the first-year medical students based on biochemical evaluation of "clinical laboratory reports" of patients in the year 2014. NMC has given prominence to this concept in the revised CBME curriculum and syllabus. We have incorporated lots of case studies with related questions and answers.

This book contains 20 chapters and 24 practicals (18 quantitative and 6 qualitative), with various types of questions with answers: MCQs, long answer, short answer, table viva voce, practical viva voce and questions based on case studies. We have presented this book in four colors. It contains important colored illustrations and tables which will be useful in learning and understanding the subject matter very well.

In Chapters 19 and 20, we have included the requirements of biochemistry practicals and all the practicals prescribed by NMC. Chapter 19: Medical biochemistry laboratory basic requirements, principles and procedures, covers the complete information on all the laboratory requirements, including basic reagents, glassware, equipment, instruments, various types of techniques, laboratory mathematics, and laboratory safety.

In Chapter 20, all the laboratory experiments are provided with clinical significance, normal range, instructions given to the patient, specimen type, collection method and standard operation procedures (SOPs). These experiments are safe and used in current laboratory practice. SOPs are based on manual as well as semi-automated methods and are presented with standard components of the reagents. Students will be able to perform practicals easily and quickly by referring to the SOPs.

Based on the information given in this book, students will be able to adapt to safety precautions right from the first day of working in a biochemistry laboratory. Students will be able to select appropriate requirements by referring to SOPs. Students will also be able to understand the principles of various conventional techniques and the use of routine and specialized laboratory tests and basic instrumentation.

The introduction of theory and practicals together will give a boost to the various abilities of students such as thinking, innovation and creativity.

Students working on a specific project or involved in basic research work require good information on specimen collection, preservation, transportation, factors affecting specimen quality and quality control aspects, report presentation and evaluation of reports of laboratory tests. We have given this information in Chapters 19 and 20.

This book will also be useful as a standard book of biochemistry for students of dentistry, nursing, optometry, biomedical sciences, physiotherapy, biotechnology, paramedical courses, biomedical sciences, graduate and postgraduate students of biochemistry, etc.

We look forward to suggestions from students and teachers for further updating this book.

Praful B Godkar

Darshan Praful Godkar

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Praful B Godkar

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BI7.6	Describe the antioxidant defense systems in the body	13	289
PE13.1	Describe the RDA, dietary sources of iron, and their role in health and disease	13	290
PE13.3	Describe the causes, diagnosis, and management of iron deficiency	13	291
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PA16.1	Define and classify hemolytic anemia	13	292
PA16.4	Describe the pathogenesis and clinical features and hematological indices and peripheral blood picture of acquired hemolytic anemia.	13	293
PA16.2	Describe the pathogenesis and clinical features and hematological indices of hemolytic anemia.	13	293
PA16.3	Describe the pathogenesis and clinical features and peripheral blood picture of sickle cell anemia and thalassemia.	13	295
PA25.1	Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, distinguish between direct and indirect hyperbilirubinemia	13	297
BI8.1	Discuss the importance of various dietary components and explain the importance of dietary fiber	14	306
BI8.5	Summarize the nutritional importance of commonly used items of food including fruits and vegetables (micromolecules and their importance)	14	314
PE9.3	Explain the calorific value of common Indian foods	14	314
BI8.3	Provide dietary advice for optimal health in childhood and adulthood, in the diseases like diabetes mellitus, coronary artery disease, and in pregnancy	14	315
PA12.2	Describe the pathogenesis of disorders caused by protein calorie malnutrition and starvation.	14	320
PE9.1	Describe the age-related nutritional needs of infants, children, and adolescence including micronutrients and vitamins	14	320
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PE10.1	Define and describe the etiopathogenesis, classify including WHO classification, clinical features, complications and management of severe acute nourishment (SAM) and moderate acute nourishment (MAM)	14	322
PE10.2	Outline the clinical approach to a child with severe acute nourishment (SAM) and moderate acute nourishment (MAM)	14	322

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PE11.1	Describe the common etiology, clinical features and management of obesity in children	14	326
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SU1.1	Describe basic concept of homeostasis, enumerate the metabolic changes in injury and their mediators	14	332
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BI7.1	Describe the structure and functions of DNA, and RNA, and outline the cell cycle	15	337
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PA25.1	Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, distinguish between direct and indirect hyperbilirubinemia	17	389
PY8.4	Describe function tests of the thyroid gland, adrenal cortex, adrenal medulla, and pancreas	17	398
PY4.9	Discuss the physiological aspects of peptic ulcer, gastro-oesophageal reflux disease, vomiting, diarrhea, constipation, adynamic ileus, and Hirschsprung's disease	17	414
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BI10.5	Describe antigens and concepts involved in vaccine development	18	439
PE19.3	Vaccine description with regards to the classification of vaccines, strain used, dose, route, schedule, risks, benefits, side effects, indications, and contraindications	18	439
PE19.2	Explain the epidemiology of vaccine-preventable diseases	18	441
PE19.1	Explain the components of the universal immunization program and the subnational immunization program	18	442
PE19.4	Define cold chain and discuss the methods of safe storage and handling of vaccines	18	447
PE19.5	Discuss immunization in special situations: HIV-positive children, immunodeficiency, preterm, organ transplants, those who receive blood and blood products, splenectomized children, adolescents, and travellers	18	448
BI11.19	Outline the basic principles involved in the functioning of instruments commonly used in a biochemistry laboratory and their applications	19	452
BI11.6	Describe the principles of colorimetry	19	458
BI11.18	Describe the principles of spectrophotometry	19	458
BI11.1	Describe commonly used laboratory apparatus and equipment, good safe laboratory practice, and waste disposal	19	460, 469
BI11.2	Describe the preparation of buffers and estimation of pH	19	462

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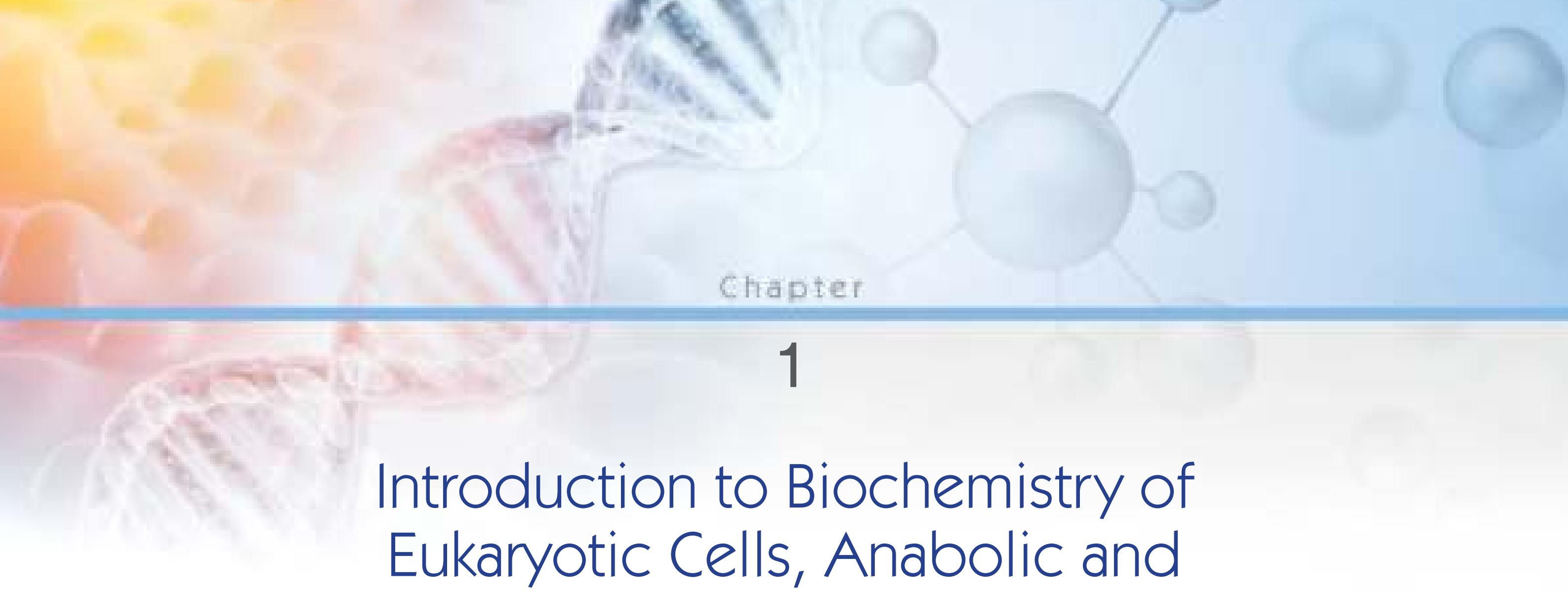
BI11.7	Demonstrate creatinine clearance	20	496
BI11.13	Demonstrate estimation of SGPT and SGOT	20	498
BI11.14	Demonstrate estimation of serum alkaline phosphatase	20	501
BI11.12	Demonstrate estimation of serum bilirubin	20	503
BI11.9	Demonstrate estimation of serum cholesterol and HDL-cholesterol	20	505
BI11.10	Demonstrate estimation of serum triglycerides	20	506
BI11.11	Demonstrate estimation of serum calcium and inorganic phosphorus	20	508
BI11.8	Demonstrate estimation of serum proteins, albumin, globulins, and A/G ratio	20	510
BI11.15	Describe and discuss the normal composition of CSF	20	512
BI11.3	Describe the chemical composition of normal urine	20	513
BI1.16	Observe working of a biochemistry autoanalyzer	20	519



Chapter

1

Introduction to Biochemistry of Eukaryotic Cells, Anabolic and Catabolic Reactions

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each

INTRODUCTION

SAQ: Define biochemistry.

Ans: Biochemistry is a science that deals with the chemical constituents of living cells, including the biochemical reactions and processes in living organisms. Biochemistry governs all living organisms and living processes. The study of biochemistry is essential to understand medicine as well as all the subjects of life sciences.

SAQ: Define medical biochemistry.

Ans: Medical biochemistry is the branch of biochemistry that studies the chemical compositions and metabolic reactions in the human body as well as the changes that take place in chemical processes and metabolic reactions in various disorders and diseases and their effects on the general health of a person. Medical biochemistry has an enormous impact on the understanding and maintenance of health and understanding and effective treatment of diseases.

SAQ: Define clinical biochemistry.

Ans: Clinical biochemistry deals with biochemistry laboratory applications to find out the cause of a disease.

Importance of Clinical Biochemistry

BAQ: Write a brief on the importance of clinical biochemistry.

Ans: The chemical constituents of various body fluids such as blood serum, plasma, urine, cerebrospinal fluid (CSF), and other body fluids are analyzed in a clinical biochemistry laboratory. These determinations are useful in diagnosing various clinical conditions such as diabetes mellitus, jaundice, gout, hyperlipidemia, hypo- and hyperthyroidism, pancreatitis, rickets, etc. Biochemistry tests are very useful in determining the severity of diseases related to many organs, such as the heart, kidneys, lungs, liver, stomach, brain, etc., as well as the endocrine disorders and related status of acid–base balance of the body. The clinical biochemistry tests, concerning the various clinical conditions, can be useful to reveal the causes of the diseases and suggest effective treatment. The biochemistry test reports can assist in monitoring the progress of a pathological condition and help in assessing response to therapy.

Early clinical exposure: For the students of medicine, the medical biochemistry subject is an extremely important pre-clinical subject along with anatomy and physiology for the "early clinical exposure concept" introduced by the National Medical Commission.

The knowledge acquired in medical biochemistry will be useful for the students to integrate molecular events with the structures and functions of the human body in health and disease.

EUKARYOTIC CELL (Fig. 1.1)

Competency achievement: The student should be able to:

BI1.1: Describe the molecular and functional organization of a cell and its subcellular components

BAQ: Briefly describe the nucleus of a cell.

Ans: The nucleus of a cell is surrounded by a double membrane. It contains a colloidal solution of proteins with various salts called nucleoplasm. It is acidic in nature.

The nucleus contains the important nucleic acid, deoxyribonucleic acid (DNA), which is associated with histone (protein) to form nucleosomes. Most of this protein is bound to DNA. The nucleus generally contains one or two refractile particles called nucleoli. Nucleoli are not membrane-bound structures. In nucleoli, ribosomal RNA (rRNA) is synthesized according to the directions from the DNA.

The outer membrane of the nucleus exhibits nuclear pores, which are open to the cytoplasm. Through these pores, contact with the cytoplasmic (or endoplasmic) reticulum is possible. The size of the nucleus is about 6 μm.

SAQ: What are the functions of DNA?

Ans: Functions of DNA:

A. DNA plays a very important role in cell division and transmission of hereditary characteristics.

B. With the help of various forms of RNA, it also plays an important role in the synthesis of various proteins.

SAQ: Briefly describe the cytoplasm.

Ans: The cytoplasm is a watery and homogeneous solution of protein, sugars, and various salts. It is basic in nature.

The cytoplasmic fluid has osmotic pressure equal to 0.9 g/dl sodium chloride (normal saline). It contains: (1) Cytoplasmic (endoplasmic) reticulum, (2) Golgi apparatus, (3) mitochondria, (4) centrosome, (5) lysosome, and (6) cytoplasmic inclusions.

SAQ: What is a cytoplasmic (endoplasmic) reticulum?

Ans: It is a complicated system of internal membranes lined by dots that are rich in RNA and called ribosomes.

SAQ: What are the important functions of the cytoplasmic (endoplasmic) reticulum?

Ans: Following are important functions of the cytoplasmic (endoplasmic) reticulum:

A. The free ribosomes are involved in the synthesis of proteins necessary for the cell itself.

B. The ribosomes attached to the membrane play an important role in the synthesis of various proteins secreted by the cell.

SAQ: What is the Golgi apparatus?

Ans: The Golgi apparatus is an organelle found in the cytoplasm of most eukaryotic cells. It forms a part of the cellular endomembrane (group of membranes) system.

SAQ: What is an endoplasmic reticulum?

Ans: The endoplasmic reticulum is a continuous membrane system in the form of a series of flattened sacs within the cytoplasm of eukaryotic cell. There are two types: Rough endoplasmic reticulum containing ribosomes, attached to the outer surface and smooth endoplasmic reticulum that does not contain ribosomes (Fig. 1.1). The endoplasmic reticulum is situated adjacent to the cell nucleus, and its membrane is continuous with the outer membrane of the nuclear envelope.

SAQ: What are two important functions of the endoplasmic reticulum?

Ans: The following are two important functions of the endoplasmic reticulum:

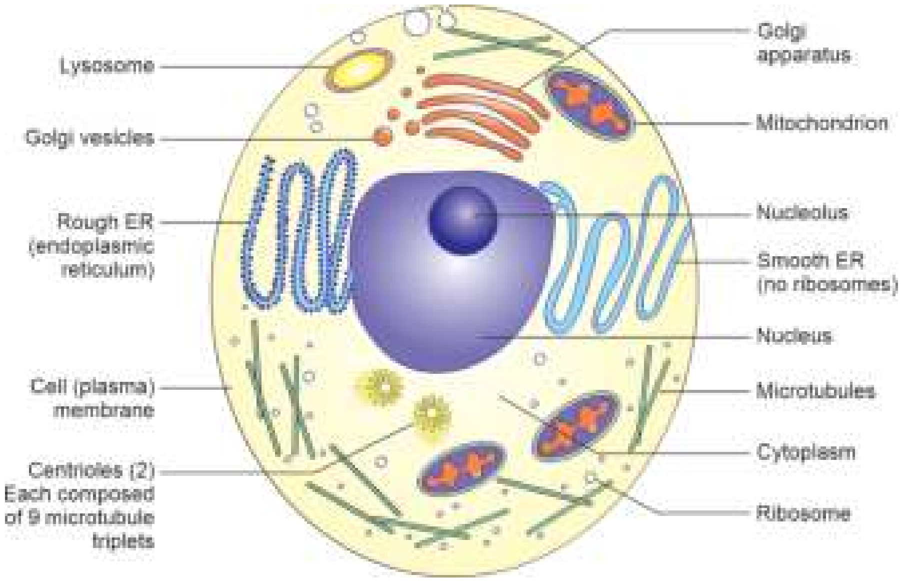

Lysosome
Golgi vesicles
Rough ER (endoplasmic reticulum)
Cell (plasma) membrane
Centrioles (2) Each composed of 9 microtubule triplets
Golgi apparatus
Mitochondrion
Nucleolus
Smooth ER (no ribosomes)
Nucleus
Microtubules
Cytoplasm
Ribosome

Fig. 1.1: Eukaryotic cell

1. Ribosomes of rough endoplasmic reticulum play an important role in the synthesis of proteins, which are modified and transported to Golgi bodies for further modifications, sorting and processing.
2. The smooth ER is involved in the synthesis of lipids such as cholesterol and phospholipids.

BAQ: Describe the composition of the Golgi apparatus.

Ans: The Golgi apparatus is composed of stacks of membrane-bound structures known as cisternae. A mammalian cell contains approximately 40 to 100 such stacks. Each cisterna appears like a flat disc enclosed by a membrane.

The two sides of a Golgi stack are referred to as a *cis* face, which means a receiving part near the endoplasmic reticulum (ER) and a *trans* face means a shipping part away from the ER. The transport vesicles from the ER carry material to the *cis* face from where they enter the Golgi and after getting processed and packaged into vesicles. Then they are shipped out from the *trans* face to the various parts of the cell.

BAQ: What are the functions of the Golgi apparatus?

Ans: Functions of Golgi apparatus:

A. Golgi apparatus primarily modifies proteins delivered from the rough endoplasmic reticulum.

B. It is also involved in the transport of lipids around the cell and the creation of lysosomes.

C. The cisterna contains special Golgi enzymes which modify cargo proteins that travel through it. Enzymes within the cisternae can modify the proteins by the addition of carbohydrates and phosphates by importing substances such as nucleotide sugars from the cytosol. These modifications also form a signal sequence that determines the final destination of the protein.

SAQ: Describe mitochondria briefly.

Ans: Mitochondria are fluid-filled vessels enclosed by inner involuted membrane folds (cristae) and a single outer layer of the protein molecule, which is internally lined by phospholipid molecules. These are minute bodies scattered throughout the cytoplasm. The components of the electron transport chain and oxidative phosphorylation are present in the inner mitochondrial membrane. The average size of mitochondria is about 0.5–10 μm.

BAQ: What are the functions of mitochondria?

Ans: Functions of mitochondria:

A. The internal chamber of mitochondria (matrix) contains various enzymes which participate in the metabolism of amino acids, lipids, and carbohydrates.
B. The mitochondria are power plants that are responsible for the process of respiration and phosphorylation in the cell, which involve the interaction of many enzymes and coenzymes. They supply energy for metabolic reactions in the form of ATP.
C. The mitochondrial matrix also contains circular double-stranded DNA, RNA, and ribosome, which are responsible for the synthesis of mitochondrial protein.

SAQ: What are centrosomes (centriole)?

Ans: These are small refractile bodies (one or two) found near the nucleus of most living cells in condensation of protoplasm.

SAQ: What are the functions of centrosomes?

Ans: During mitosis, the two centrioles move to the opposite poles of the cell and support skin with fine protoplasmic rays. Along this spindle, the chromosomes arrange themselves after division.

SAQ: What are peroxisomes and their functions?

Ans: Peroxisomes are spherical or oval single-membrane cellular organelles.

The functions of the peroxisomes: Peroxisomes contain the enzyme catalase, which protects the cell from toxic substances produced during metabolic reactions.

SAQ: Describe briefly the cytoskeleton of a cell.

Ans: The cytoskeleton of a cell is in the form of a complex mesh of protein filaments. It includes microfilaments and microtubules. It extends throughout the cell cytoplasm.

SAQ: What are the important functions of the cytoskeleton?

Ans: Functions of cytoskeleton:

A. It maintains the shape of the cell.
B. It controls the position of cell organelles by anchoring them to the plasma membrane.
C. It is involved with cytoplasmic streaming, which means the flow of the cytoplasm.
D. It anchors the cell properly by interacting with extracellular elements.

SAQ: What are the microtubule organizing centers (MTOCs) of a cell and their functions?

Ans: Centrioles and centrosomes are the non-membranous structures that are present outside the nuclear membranes.

SAQ: What are the functions of MTOCs?

Ans: MTOCs organize spindle fibers and give rise to the formation of spindle apparatus which is required for cell division.

BAQ: Briefly describe cytoplasmic membrane and its functions.

Ans: The cell is bounded by an approximately 10 nm thick, double-layered, semipermeable membrane. It is made up of protein and lipid components.

Functions of cell membrane:

A. *Exchange of food and secretory products:* Cytoplasmic membrane conducts the exchange of food and secretory products in the following ways: Passive transport: When the movement of substances is under the influence of osmotic pressure.

   Active transport: When the movement of substances is mediated by chemical interactions and electrical changes.

B. *Pinocytosis:* The cytoplasmic membrane plays an important role in pinocytosis (cell drinking) and phagocytosis (cell eating).
C. *Signal transduction:* It plays a very important role in the signal transduction

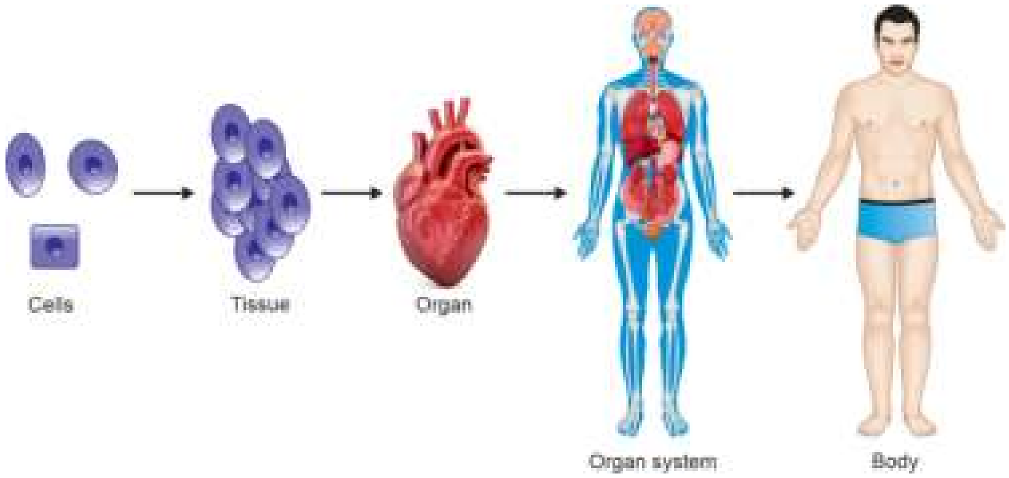


Fig. 1.2: Basic levels of body organization

process using one membrane-bound enzyme adenylate cyclase, which synthesizes cyclic AMP (cAMP) from ATP. A specific membrane protein changes shape and relays the message inside the cell.

D. *Cell-to-cell recognition:* Specific glycoproteins present on cell membranes play an important role as recognition molecules by other cells.

E. Cell-to-cell attachments: Various proteins like desmosomes play an important role in attachment to the cytoskeleton and extracellular matrix. This is useful in maintaining cell shape and stability.

BASIC LEVELS OF BODY ORGANIZATION

BAQ: Utilizing a line diagram show basic levels of body organization.

Ans: Figure 1.2 shows basic levels of body organization formed from cells to tissues to organs to the body.

CELLULAR INJURY AND DEATH

BAQ: Write a brief on cellular injury and death.

Ans: The normal cellular function requires a balance between the physiological demands of a cell and its metabolic capability, which maintains a state of homeostasis, which means maintenance of a stable and constant condition of properties like temperature and pH. Cells can alter their functional state in response to any stress to maintain homeostasis by undergoing hypertrophy (increase in mass) or hypotrophy (decrease in mass). Cell injury can be averted if any situation that leads to stress is checked.

Irreversible injury to the cells can lead to pathological changes leading to cellular death. Cellular injury can be caused by the following factors: Nutritional imbalances (related to protein calories, vitamins, etc.), infectious agents (e.g. bacteria, viruses), chemical agents (drugs and nontherapeutic agents), physical agents (radiations, electric shock, trauma, heat, cold, etc.), adverse immunological reactions, genetic derangements, and oxygen deprivation. There are following two morphological patterns of cellular death—

1. *Necrosis:* It is a type of cellular death caused by excessive cell swelling and denaturation of proteins, leading to cellular rupture.
2. *Apoptosis:* It occurs when a cell dies as an internal program organized to eliminate unwanted cells produced during embryogenesis and pathological conditions, without disrupting a normal tissue.

SAQ: Define necrosis and apoptosis.

Ans: Refer to upper paragraphs.

SAQ: Give an example of how a damaged cell leads to a specific disease.

Ans: Due to COVID-19 infection of lung cells, the infection can spread to the lung tissue and severe infection can affect many organs such as the liver, kidneys, blood cells, and ultimately the entire body of an individual.

METABOLIC REACTIONS

Life is a chemical process that involves thousands of various types of chemical reactions occurring in an organized manner. These are metabolic reactions and are collectively called metabolism.

SAQ: Define metabolism.

Ans: Metabolism is defined as the total of all chemical reactions that occur in the body, which is a sum of catabolism and anabolism.

SAQ: Define catabolism.

Ans: Catabolism is that part of metabolism that involves the breakdown of large, complex molecules into smaller, more simplified products.

SAQ: Describe catabolism.

Ans: Catabolism takes place during digestion, removal of hydrogen (dehydrogenation), carboxyl groups (decarboxylation) and amino groups (deamination), oxidation, etc. Catabolic rections are energy-yielding. ATP molecules are obtained by the main catabolic reactions, such as the breakdown of glucose, amino acids, and fatty acids.

Catabolic reactions are energy-yielding. They are involved in the breakdown of more complex molecules into simpler ones.

SAQ: Describe anabolism.

Ans: Anabolism is that part of metabolism that involves the synthesis of larger, more complex molecules from small, simple reactants. Examples of anabolism include the synthesis of glycogen from glucose, protein from amino acids, fat from glycerol and fatty acids, and the construction of new antibodies and new enzymes. Anabolic reactions require energy.

Rate-Limiting and Committed Steps in Metabolism

Metabolic reactions are controlled according to the requirements of a cell. For example, in a fed state, when plenty of glucose is available to poduce ATP molecules, glycolysis (breakdown of glucose) will continue till sufficient ATP molecules are produced, and later on, other metabolic reactions will start to store excess glucose into glycogen and fat.

Metabolic reactions (pathways) require strict regulations so that the proper compounds get produced in the proper amounts. Such regulations ensure that the products formed in metabolic reactions do not accumulate and lead to a situation that can be wasteful or even harmful to the cell. A rate-limiting reaction is a slow reaction that controls the rate of reactions of metabolism by decreasing the speed of a metabolic pathway. In every metabolic pathway, there is at least one enzyme-catalyzed reaction that is “rate-limiting”, due to the low concentration of reaction catalyzing “enzyme”. Rate-limiting reaction is not dependent on the specific substrate (a substance on which an enzyme acts) but it is dependent on the concentration of the reaction-catalyzing enzyme.

A committed step in metabolic pathways (reactions) is an effective first irreversible enzymatic reaction. After this step, the metabolic reactions end up in the required “final product” (e.g. the formation of ATP molecules at the end of glycolysis).

NOTE

1. Often, the first committed step is regulated by processes such as feedback inhibition and activation of enzymes.
2. Rate-limiting steps are different from committed steps. However, in a specific metabolic pathway, a rate-limiting step can also be a committed step, e.g. in the case of glycolysis Step 3 is both a rate-limiting as well as committed (Fig. 4.5, p 45).

Homeostasis

Competency achievement: The student should be able to:

SU1.1: Describe the basic concept of homeostasis

BAQ: Describe the basic concept of homeostasis.

Ans: Homeostasis is a state of steady internal balance among all the body systems needed for the body to survive and function normally. In homeostasis, the body's levels of energy, oxygen, blood pressure, blood sugar, proteins, blood buffers, electrolytes, hormones, proteins, and temperature are constantly adjusted to respond to changes inside and outside the body, to keep them at normal levels.

All the homeostatic control mechanisms have a minimum of three interdependent components for the regulation of changes that may disturb homeostasis:

1. A receptor,
2. A control center, and
3. An effector.

1. A receptor acts as a sensing component and it monitors and responds to changes in the environment, either internal or external. Examples of receptors are mechanoreceptors and thermoreceptors. Mechanoreceptors are a specific type of somatosensory receptors that relay extracellular stimulus to intracellular signal transduction. A thermoreceptor is a sensory receptor, the receptive portion of a sensory neuron that senses absolute and relative changes in temperature.
2. Control centers include the renin–angiotensin system and the respiratory center. The renin–angiotensin system is a hormone-regulated system that regulates blood pressure, body fluids, and electrolyte balance.
3. An effector acts to bring about the change back to the normal state. At the cellular level, effectors are the nuclear receptors that bring about changes in gene expression through down-regulation or up-regulation of specific processes and act in negative feedback mechanisms.

The following are the main components of the homeostasis action:

1. *Receptor:* Cutaneous receptors of the skin senses high humidity and temperature
2. *Control center:* The brain acts by the action of the nervous system.
3. *Effector:* Causes vasodilation of blood vessels to drop body temperature and secretion of sweat through sweat glands in the skin and evaporation of sweat on the surface of the skin to keep the body cool. If the external temperature is too cold, the blood vessels constrict (vasoconstriction) and the body can retain heat.

Homeostasis breakdown: The failure of homeostasis due to accidents or severe disease may lead to disability and death. The following are the common factors that may affect homeostasis: Physical condition, nutrition, toxins, and adverse effects of medicines and medical procedures.

Competency achievement: The student should be able to relate:

Horizontal and vertical integration of subjects

BAQ: What is the meaning of horizontal integration of a specific biochemical topic with other pre-medical subjects such as anatomy, physiology, and nutrition? Give examples.

Ans: Horizontal integration of a specific biochemical topic with other pre-medical subjects is useful to understand the effects of a specific biochemical change on anatomical and physiologic aspects of an individual and support of nutrition to get rid of the biochemical change.

For example, when the iron is deficient, normal formation of red blood cells will not take place (anatomical effect), the oxygen-carrying capacity of the body will decrease (physiological change), and by nutritional treatment (nutritional therapy), supported by iron tonics, and treating the root cause of the disease, it is possible to get rid of iron deficiency.

BAQ: What is the meaning of vertical integration of a specific biochemical topic with other paramedical subjects such as microbiology and pharmacology?

Ans: Vertical integration of a specific biochemical topic with other paramedical subjects such as microbiology and pharmacology is useful to understand; whether the effects of a specific biochemical change are related to microbial infection. Vertical integration with pharmacology will give an idea about the study of the specific drug to treat microbial disease.

Example: In the case of a patient suffering from severe diarrhea, the biochemical change is the loss of water and electrolytes from the gastrointestinal tract. Identification of the specific microorganism and use of appropriate antibiotics will be useful to control diarrhea and loss of water and electrolytes.

Multiple Choice Questions

Q1. Which of these are located in the mitochondria?

A. Cytochrome oxidase
B. Succinate dehydrogenase
C. Amylase
D. A and B

Q2. The most active site of protein synthesis is

A. Nucleus B. Ribosome
C. Mitochondrion D. Cell sap

Q3. Mitochondrial DNA is

A. Circular double-stranded
B. Circular single-stranded
C. Linear double helix
D. None of these

Q4. Which of the following cellular organelles are called "suicide bags"?

A. Lysosomes B. Ribosomes
C. Nucleolus D. Golgi bodies

Q5. The Golgi complex

A. Synthesizes proteins
B. Produces ATP
C. Provides a pathway for transporting chemicals
D. Forms modified components like glycoproteins

Q6. The following points about microfilaments are true *except*

A. They form cytoskeleton with microtubules
B. They provide support and shape
C. They form intracellular conducting channels
D. They are involved in muscle cell contraction

Q7. The following substances are cell inclusions *except*

A. Melanin B. Glycogen
C. Lipids D. Vitamins

Q8. Enzymes catalyzing electron transport are present mainly in the

A. Ribosomes
B. Endoplasmic reticulum
C. Lysosomes
D. Inner mitochondrial membrane

Q9. rRNA is produced mainly in the

A. Endoplasmic reticulum
B. Ribosome
C. Nucleolus
D. Nucleus

Q10. Genetic information of nuclear DNA is transmitted to the site of protein synthesis by

A. rRNA B. mRNA
C. tRNA D. Polysomes

Q11. The powerhouse of the cell is

A. Nucleus
B. Cell membrane
C. Mitochondria
D. Lysosomes

Q12. The digestive enzymes of cellular compounds are confined to

A. Lysosomes B. Ribosomes
C. Peroxisomes D. Polysomes

Q13. Which group is involved in manufacturing substances needed by the cell?

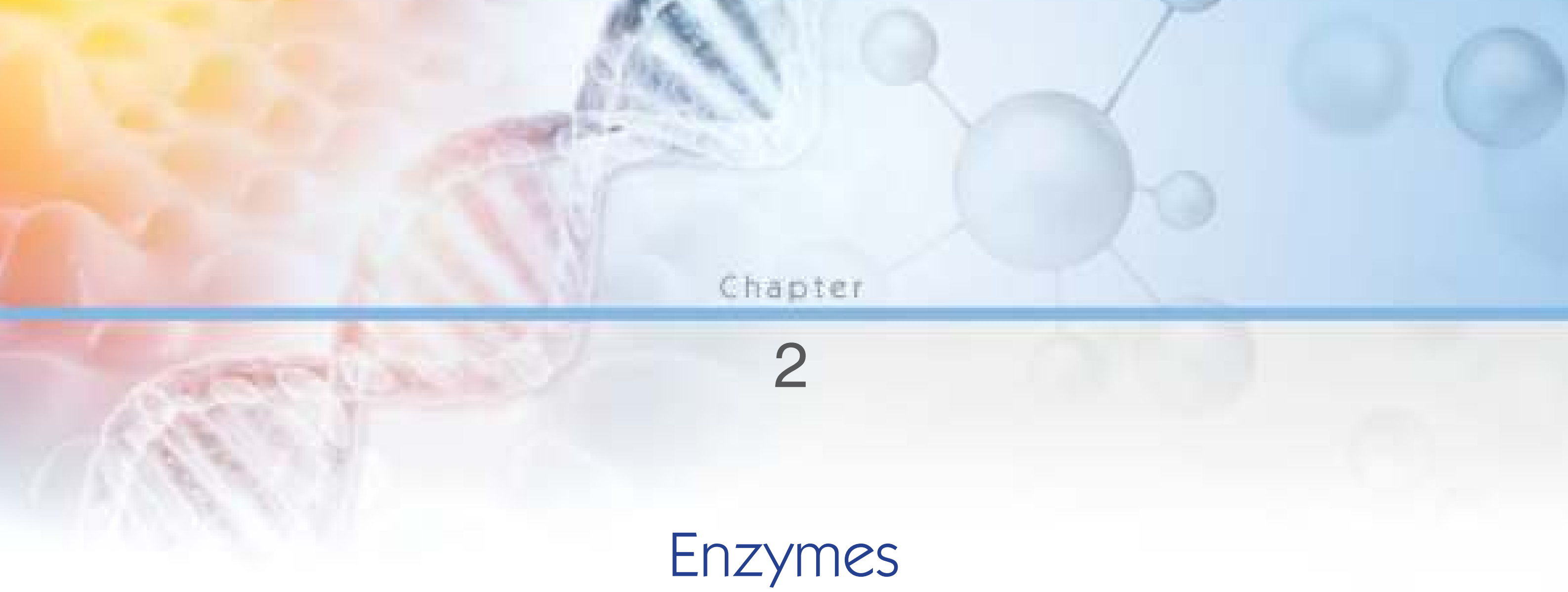
A. Lysosome, vacuole, ribosome
B. Ribosome, rough ER, smooth ER
C. Vacuole, rough ER, smooth ER
D. Smooth ER, ribosome, vacuole

Q14. Which of the following clues would tell you whether a cell is prokaryotic or eukaryotic?

A. The presence or absence of a rigid cell wall
B. Whether or not the cell is partitioned by internal membranes
C. The presence or absence of ribosomes
D. Whether or not the cell contains double-stranded DNA

Answers

1. D **2.** B **3.** A **4.** A **5.** D **6.** C **7.** D **8.** D **9.** C **10.** B
11. C **12.** A **13.** B **14.** D



Chapter

2

Enzymes

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

Competency achievement: The student should be able to:

BI2.1: Explain fundamental concepts of enzyme, isoenzyme, alloenzyme, coenzyme, and co-factors. Enumerate the main classes of IUBMB nomenclature

INTRODUCTION

BAQ: Write a brief note on enzymes.

Ans: Enzymes are synthesized by the cells of all living organisms. They act like catalysts and accelerate the multitude of metabolic reactions carried out by the cells. All enzymes are proteins, and their catalytic activity depends on the presence of a precise conformational structure in the folded polypeptide chains. Even small alterations in this structure may result in the loss of enzyme activity. The enzymes act on specific substrates. The substrate is a substance on which an enzyme acts and specific end products are produced. In health, all physiologic processes occur in an ordered, regulated manner mainly due to the catalytic functions of enzymes. In living organisms, enzymes are rapidly degraded, and their supply is replenished by new synthesis.

Enzymes play a very important role as catalysts to carry out processes such as digestion, absorption, respiration, neurotransmission, coagulation, acid–base balance, synthesis of various proteins, lipoproteins and hormones, and several other anabolic reactions, energy-providing catabolic reactions, and defense mechanisms, reactions involving the transportation of various substances and muscular activities.

Homeostasis can be severely disturbed in various clinical conditions, which can impair the ability of cells to form enzymes. For example, in liver cirrhosis, since cells are unable to synthesize key enzymes responsible to convert ammonia into urea, ammonia intoxication occurs. Several genetic diseases are linked to the absence of certain important enzymes, which participate in the synthesis of metabolites.

Following severe tissue injury specific tissue enzymes are released into the blood. Measurement of these intracellular enzymes in serum provides physicians with valuable diagnostic and prognostic information.

In diagnostic technology, it was possible to introduce enzyme-based reagents, that are nontoxic, noncorrosive, and very safe to handle. The ability of enzymes to conjugate with other proteins without significant loss of catalytic activity has been used in various types of ELISA methods. The enzyme labels are used instead of radioactive isotopes in ELISA techniques for the determination of various hormones, antibodies, and drugs in a clinical chemistry laboratory.

BAQ: Define enzymes and their properties and functions.

Ans: Enzymes are proteins in nature and act as biological catalysts and help body cells to carry out biochemical reactions.

Properties of Enzymes

A. Enzymes are proteins. Hence, they get denatured at extremely high temperatures and pH

B. Enzymes are stable in normal saline

C. Enzymes act as catalysts

D. Enzymes are specific.

E. Enzyme activity depends on the concentration of the substrate.

Functions

The enzymes help to carry out various catalysis reactions. In the catalysis process, the enzyme acts on a specific substrate by increasing the rate of a chemical reaction. Like all other catalysts, the enzyme is not consumed in the reaction and remains unchanged at the end of the reaction.

SAQ: What is a substrate? Give two examples.

Ans: Substrate is a substance on which an enzyme acts.

Example 1: Amylase acts on starch (substrate) and the end products are dextrins and maltose.

Example 2: Lipase acts on lipids (substrate) and the end products are glycerol and fatty acids.

Enzyme Catalysis

SAQ: What are the various processes in which enzymes play a role as a catalyst?

Ans: Enzymes play a very important role as catalysts to carry out processes such as digestion, absorption, acid–base balance, respiration, synthesis of various proteins, lipoproteins and hormones, and several other anabolic reactions, energy-providing catabolic reactions, neurotransmission, coagulation, and defense mechanisms, reactions involving the transportation of various substances and muscular activities.

BAQ: Give information on the initial rate of reaction of an enzyme-catalyzed reaction, the Michaelis-Menten equation, and the K_m value.

Ans: Initial rate of reaction of an enzyme-catalyzed reaction is directly proportional to the concentration of the substrate and also to the concentration of the enzyme. However, the initial rate of reaction decreases when the concentration of substrate and enzyme is increased, respectively (Fig. 2.1).

The substrate concentration that produces half the maximum velocity is the K_m value. Also known as the Michaelis constant (Fig. 2.1).

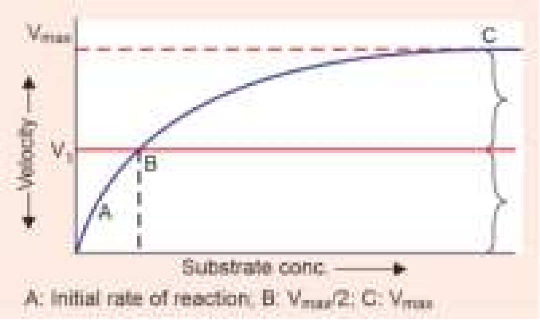


Fig. 2.1: Effect of substrate concentration on enzyme activity

The Michaelis-Menten equation is expressed as follows:

$$V_i = \frac{V_{max}[S]}{K_m + [S]}$$

V_i = Initial velocity of the reaction
V_{max} = Maximum velocity of the reaction
S = Substrate concentration

SAQ: Why even minor alterations in enzyme structure may result in the loss of its activity?

Ans: All the enzymes are proteins, and their catalytic activity depends on the presence of a precise conformational structure in the folded polypeptide chains. Even minor alterations in this structure may result in the loss of enzyme activity.

COENZYMES

BAQ: What are coenzymes? Give four examples of coenzymes and one enzyme-catalyzed reaction that requires a coenzyme.

Ans: Coenzymes are non-protein organic substances required for some enzymes for their full activities. No catalysis will take place unless both enzyme and coenzyme are present.

The complete system in which coenzymes are required is called a holoenzyme. It contains an enzyme (protein) and coenzyme, a dialyzable, nonprotein organic substance. The reactions involving group transfer, oxidation–reductions, conversion of isomers, and the reactions resulting in the formation of covalent bonds frequently require coenzymes.

Coenzymes frequently contain B complex vitamins as part of their structure. The B-complex vitamins, nicotinamide, thiamine, riboflavin, lipoic acid, and pantothenic acid are important constituents of coenzymes for biological oxidation and reduction. Folic acid and carbamide coenzymes function in one-carbon metabolism (biochemical reactions involving the transfer and utilization of a single carbon).

The following are two classes of coenzymes based on functional characteristics:

A. Coenzymes for group transfer of groups other than 'H'
    1. CoA
    2. B_6 phosphate
B. Coenzymes for transfer of 'H'
    1. NAD^+, $NADP^+$
    2. FMN, FAD

Example: Chemical reaction catalyzed by lactate dehydrogenase (LDH) using NAD as coenzyme:

$$\text{Lactic acid} + \text{NAD} \xrightleftharpoons{\text{LDH}} \text{Pyruvic acid} + \text{NADH}$$

ISOENZYMES

BAQ: What are isoenzymes? Give three examples.

Isoenzymes are physically distinct forms of the same enzyme, which have the same catalytic activity. They, thus, catalyze the same reaction.

Although they possess identical or very similar activity, the enzymes from different sources are not identical proteins since they may differ in physical, biochemical, and immunological properties. Due to the difference in their molecular weights and sizes, their rates of migration in the electrophoresis procedure are found to be different. They may also differ in their properties, such as stability to heat denaturation, resistance to chemical inhibiting agents, and affinity for substrates and coenzymes.

Examples of Isoenzymes

The following five isoenzymes of lactate dehydrogenase are LD_1, LD_2, LD_3, LD_4 and LD_5.

Cardiac muscle is the richest in LD_1 and liver muscle is the richest in LD_5. The kidney contains a high proportion of LD_2.

The following are isoenzymes of creatine phosphokinase (CK): CK-MM (present in muscles), CK-BB (present in the brain), and CK-MB (present in the heart tissue).

In the case of alkaline phosphatase (ALP), the enzyme present in normal adult serum is mostly from the liver, while in children, it is a mixture of liver and bone enzymes. Other isoenzymes may originate from the kidney, placenta, and small intestine.

BAQ: What are metallic positive modifiers of enzymes? Give examples. Describe their actions.

Ans: The activity of some enzymes can be reversibly increased by small inorganic molecules termed positive modifiers. In most cases, metal ions act as positive modifiers.

Examples of the metal ions that act as positive modifiers are: Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}.

Metal may accelerate enzyme-catalyzed reactions by:

1. Direct participation in catalysis.
2. Reacting with the substrate to form a true substrate.
3. Altering the equilibrium constant of the apparent overall reactions.
4. Bringing about a conformational change in the protein by converting it from an inactive to an active conformation.
5. Combining first with the enzyme and forming a metalloenzyme (ME) which then binds with the substrate, and subsequently, a product is formed.

Many highly purified enzymes, such as catalase and alcohol dehydrogenase, contain a low, highly reproducible number of tightly bound metal ions per mol or per subunit of protein. Removal of the metal by complexion with chelating agents, such as EDTA, often results in partial or total loss of enzyme activity.

Factors that Affect Enzyme Activity (Fig. 2.2)

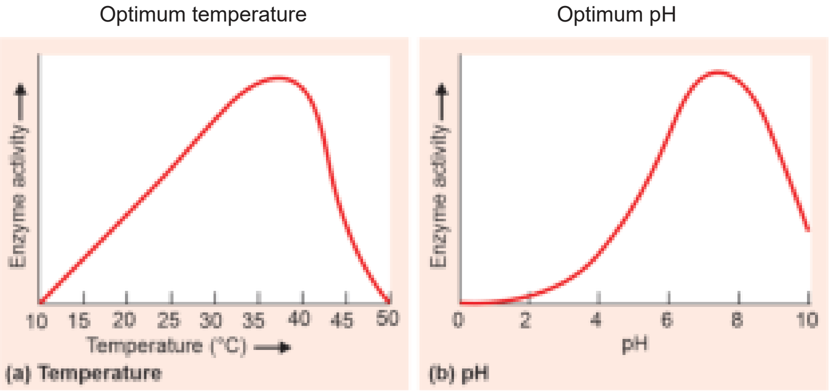
SAQ: Enumerate various factors that affect enzyme activity.

Ans: The following are various factors that affect enzyme activity:

A. Substrate concentration,
B. Enzyme concentration,
C. pH of the substrate,
D. Temperature,
E. Ionic concentration of the medium (in which enzyme is present), and
F. Radiation.

BAQ: Show by graphical presentations, the effects of temperature, pH, and substrate concentration on enzyme activity.

Ans:



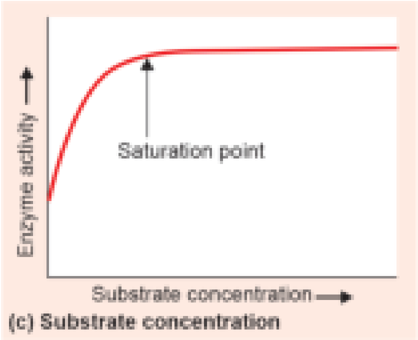


Fig. 2.2: Graphic presentation of effects of temperature, pH, and substrate concentration

ENZYME SPECIFICITY

BAQ: What is enzyme specificity? Explain with examples.

Ans: Enzymes act only on specific substrates, with a few exceptions. For example, amylase acts on starch, pepsin acts on proteins, a lipase acts on lipids, lactate dehydrogenase acts on lactic acid, etc.

Many enzymes show absolute optical specificity for at least a portion of a substrate molecule. Thus, maltase catalyzes the hydrolysis of alpha, but not beta-glycosides. Also, the enzymes of Embden-Meyerhof and direct oxidative pathways catalyze the interconversion of D, but not L-phosphosugars.

A particular enzyme acts only on a particular chemical grouping, e.g. glycosidases on glycosides, pepsin and trypsin on peptide bonds, and esterases on ester linkages.

Certain enzymes exhibit a higher order of group specificity. Chymotrypsin preferentially hydrolyses peptide bonds in which the carboxyl group is contributed by the aromatic amino acids. Carboxypeptidases and aminopeptidases split amino acids one at a time from the carboxyl or amino terminal end of polypeptide chains, respectively.

Enzyme Classification

SAQ: Enumerate various classes of enzymes based on IUBMB nomenclature:

Ans:
1. Oxido-reductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

SAQ: Give two examples of each of the following classes of enzymes: Oxidoreductase and transferases.

Ans: Oxidoreductases: Alcohol dehydrogenase and lactate dehydrogenase

Transferases: Alaninine transferase (SGPT) and aspartate transferase (SGOT)

LAQ: Describe the main enzyme classes of IUBMB nomenclature with one example each.

Ans: The function of the classification of the enzyme is to emphasize similarities and relationships in a precise manner. The International Union of Biochemists (IUB) has suggested a rational classification of enzymes that is precise, descriptive, and informative. The major features of the IUB system for the classification of enzymes are as follows:

A. The enzymes catalyzed reactions are divided into 6 major classes, each with 4 to 13 subclasses.

B. The enzyme name has 2 parts. The first is the name of the substrate/s. The second ending in ase indicates the type of reaction catalyzed.

C. Additional information may be provided to clarify the nature of enzyme-catalyzed reaction.

D. Each enzyme has a systematic code number (EC). The first digit indicates the class of the enzyme, the second digit indicates the subclass and the third digit indicates sub-subclass of the enzyme. The fourth digit is for the particular enzyme named.

Thus EC: 2.7.1.1 denotes:

- Class 2: Transferase
- Subclass 7: Transfer of phosphate
- Sub-subclass: Function of alcohol as phosphate acceptor.
- Final digit 1: The enzyme ATP, D-hexose 6-phosphotransferase.

The following are the major six classes of enzymes and related catalyzed reactions:

1. **Oxidoreductases:** Enzyme catalyzing oxidation and reductions between two substrates A and B.

$$\underset{\text{Reduced}}{A} + \underset{\text{Oxidized}}{B} \xrightleftharpoons{\text{oxidoreductase}} \underset{\text{Oxidized}}{A} + \underset{\text{Reduced}}{B}$$

Example 1: 1.1.1.1 Alcohol: NAD oxidoreductase (alcohol dehydrogenase)

Catalyzed reaction: Alcohol + NAD^+ $\rightleftharpoons$ Aldehyde (or ketone) + NADH + H^+

2. **Transferases:** Enzyme catalyzing a transfer of a group G (other than hydrogen), between a pair of substrates A and B.

   $A_{-G} + B \rightleftharpoons B_{-G} + A$

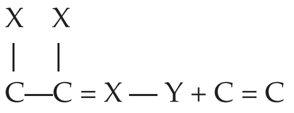
   Examples: Enzyme SGPT or ALT (alanine, alpha-ketoglutarate transferase)

   Catalyzed reaction: Alanine + alpha-ketoglutaric acid $\rightleftharpoons$ Pyruvate + Glutamate.

3. **Hydrolases:** Enzymes catalyze the hydrolysis of ether, ester, glycosyl, peptide, acid anhydride, etc. bonds.

   Examples: Enzyme pepsin that acts on peptide linkage of proteins to form peptones and proteases.

4. **Lyases:** Enzymes that catalyze the removal of groups from substrates by a mechanism other than hydrolysis, leaving double bonds. These enzymes act on C–C, C–O, C–N, C–S, and C–halide bonds.

   $$\overset{X}{\overset{|}{C}}—\overset{X}{\overset{|}{C}} = X — Y + C = C$$

   Example: 4.2.1.2 L-malate hydrolase (fumarase).

   Catalyzed reaction: L-malate $\rightleftharpoons$ fumarate + H_2O

5. **Isomerases:** This class includes all enzymes catalyzing the interconversion of optical, geometric, or positional isomers

   Example: 5.1.1.1 Alanine racemase (L-alanine isomerase)

   Catalyzed reaction: L-Alanine $\rightleftharpoons$ D-alanine

6. **Ligases:** Enzymes catalyze the linking together of 2 compounds, coupled with the breaking of a pyrophosphate bond in a compound such as ATP.

   Example: 6.3.1.2 L-glutamate ammonia, ligase (glutamine synthetase).

   Catalyzed reaction: ATP + L-glutamate + NH_4^+ $\rightleftharpoons$ ADP + ortho-phosphate

BAQ: Describe oxidoreductases under the following headings:

1. Examples
2. Catalyzed biochemical reaction
3. One related clinical condition

Ans: 1. *Example:* Lactate dehydrogenase.

2. Catalyzed biochemical reaction:

   Lactic acid + NAD $\rightleftharpoons$ Pyruvic acid + NADH

3. One related clinical condition: COVID-19 (when lungs are severely affected)

BAQ: Describe transferases under the following headings:

1. Examples
2. Catalyzed biochemical reaction
3. One related clinical condition

Ans: 1. Example: Serum glutamate pyruvate transaminase (SGPT)

2. Catalyzed biochemical reaction:

   Alanine + α-ketoglutarate $\rightleftharpoons$ Pyruvic acid + Glutamic acid

3. One related clinical condition: Infective hepatitis

BAQ: Describe hydrolases under the following headings:

1. Examples
2. Catalyzed biochemical reaction
3. One related clinical condition

Ans: 1. Example: Amylase

2. Catalyzed biochemical reaction: Starch $\rightleftharpoons$ Maltose + Dextrins

3. One related clinical condition: Acute pancreatitis

ENZYME KINETICS

Competency achievement: The student should be able to:
BI2.3: Describe and explain the basic principles of enzyme activity

LAQ: Describe and explain the basic principles of enzyme activity.

Ans: An enzyme-catalyzed reaction can be expressed in a simplified way as follows (if only one substrate is used):

$$\underset{\text{Substrate}}{S} \quad \underset{\text{Enzyme}}{\rightleftharpoons} \quad \underset{\text{Product}}{P}$$

Many enzyme-catalyzed reactions involve two substrates, and usually, two products are formed. The general formula is, therefore, written as follows:

$$\underset{(S1)\ +\ (S2)}{\text{Substrate1} + \text{Substrate2}} \overset{E}{\rightleftharpoons} \underset{(P1)\ +\ (P2)}{\text{Product1} + \text{Product2}}$$

The observations of the above enzyme-catalyzed reaction are:

1. The substrate (S) undergoes chemical change as soon as the sample containing a specific enzyme is mixed with it.
2. In the absence of enzyme (E), very little or no product (P) is formed.
3. The reaction will proceed, and the product will be formed at a rate that will depend on the concentration of E, temperature, and pH (sometimes also on the presence of coenzyme and metallic activators).
4. The chemical reaction is reversible, and the reaction may proceed in either the forward or reverse direction depending upon the equilibrium constant, which is Keq = (P)(S).
5. If the reverse reaction is being studied, the labels 'S' and 'P' are interchanged.
6. In enzyme-catalyzed reaction first enzyme-substrate complex forms ES and then product forms with unaltered enzymes.
$$S + E \rightleftharpoons ES \rightleftharpoons P + E$$
7. In a non-catalytic chemical reaction, the energy of activation is provided in the form of high temperature so that collision frequency between the molecules enables the reaction to occur. At physiological temperature (body temperature), however, due to enzyme catalysis, all the chemical reactions take place appropriately at 37°C and S gets easily converted to P.

Most of the enzyme-catalyzed reactions take place at low temperatures, at almost neutral pH, in aqueous solutions, and at low reactant concentrations, due to the following reasons:

1. Each enzyme has one or more combining sites within the active sites to which the substrates bind.
2. The binding has several effects. First, it positions substrate molecules in the most favorable relative orientations for the reaction to occur.
3. Secondly, the active site is perfectly complementary to the transition state of the substrate (and not to the original ground state of the substrate).
4. The transition state binds to the enzyme more tightly than the substrate in its ground state. The net effect is to lower the activation energy of the reaction. Once formed, the transition state rapidly converts to give products, which bind less tightly to the enzyme and diffuse away.

The rate at which an enzyme-catalyzed reaction occurs is an intrinsic property of the enzyme. Since every enzyme has a specific structure and since it undergoes a specific conformational change, it acts only on a specific substrate.

In an enzyme-catalyzed reaction, S must first be converted to the transition state, S*. It is a stage in which the electronic configuration of the substrate molecule is changed after combining with the enzyme. In this process, the activation energy of the reaction is decreased, and the product forms with an intact enzyme (Fig. 2.3).

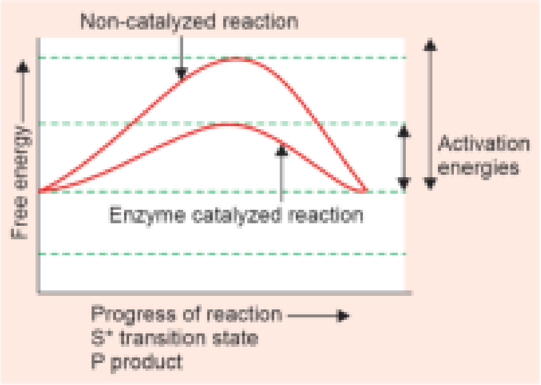


Fig. 2.3: Comparison of enzyme-catalyzed and non-catalyzed reaction

Competency achievement: The student should be able to:

BI2.4: Describe and discuss enzyme inhibitors as poisons and drugs as therapeutic enzymes

BAQ: Describe using a diagram of the working of enzymes based on induced-fit model and not based on the lock-key model.

Ans: The induced-fit model describes the action of an enzyme on a substrate. According to this system, as the substrate enters the active site of an enzyme, it induces the enzyme to alter its shape slightly and the substrate then fits appropriately, and then subsequently product and enzyme are released (Fig. 2.4).

The old lock and key model was not appropriate because it indicated that the lock and the key were unchanging.

ENZYME INHIBITION

SAQ: What are enzyme inhibitors? Give two examples.

Ans: An enzyme inhibitor is defined as a substance that binds with the enzyme and brings about a decrease in its catalytic activity. The enzyme inhibitor may be organic or inorganic.

Examples:

1. Heavy metals like mercury and lead
2. Idoacetate

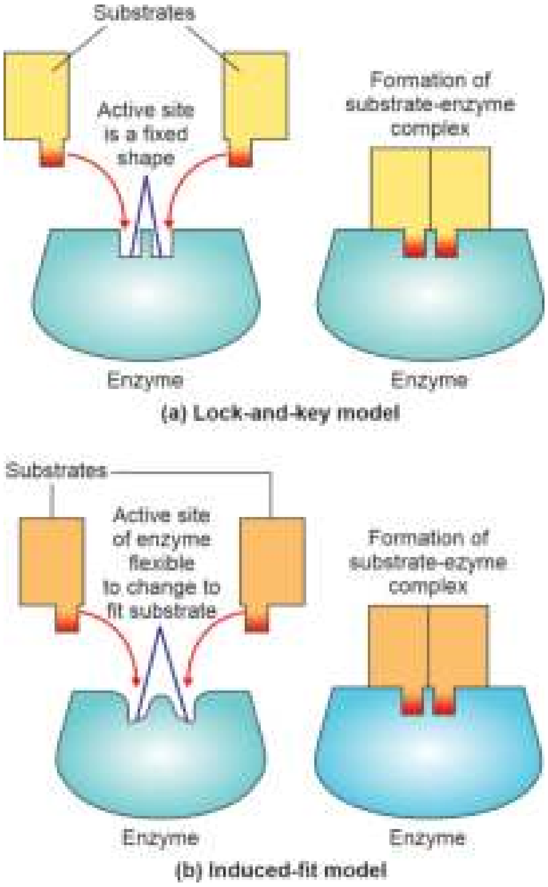

Substrates
Active site is a fixed shape
Formation of substrate-enzyme complex
Enzyme
Enzyme
(a) Lock-and-key model
Substrates
Active site of enzyme flexible to change to fit substrate
Formation of substrate-ezyme complex
Enzyme
Enzyme
(b) Induced-fit model

Fig. 2.4: Comparison of lock-and-key vs induced-fit model

SAQ: What are inorganic negative modifiers of enzymes? Give four examples.

Ans: Metals may also act as negative modifiers. The addition of metal may convert an active configuration of the enzyme into an inactive form.

Examples of inorganic negative modifiers:

Heavy metals such as mercury, lead, silver, and gold.

SAQ: Give two examples of enzyme inhibition by inorganic negative modifiers.

Ans:

1. Pyruvate dehydrogenase is also inhibited by mercury ions (mercury poisoning).
2. Succinate dehydrogenase (respiratory complex II component) is bound to

the inner mitochondrial membrane of mammalian mitochondria. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain. It is inhibited by arsenic ions.

LAQ: Describe various types of enzyme inhibitions.

Ans: Following are the various types of enzyme inhibitions:

1. Reversible inhibition
2. Irreversible inhibition
3. Allosteric inhibition

1. **Reversible inhibition:** The inhibitor binds non-covalently with the enzyme. The enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition can be (mainly):
   A. Competitive
   B. Non-competitive

   A. *Competitive inhibition* (Fig. 2.5): The inhibitor (I) resembles the substrate and competes with the substrate, and binds at the active site of the enzyme. As long as the competitive inhibitor binds at the active site of the enzyme, it is unable to act on the substrate. The inhibition could be overcome by a high substrate concentration.

   Example

   Enzyme: Succinate dehydrogenase (SDH)

   Substrate: Succinic acid

   Competitive inhibitors: Malonic acid, glutaric acid, and oxalic acid.

   The competitive inhibitors compete with succinic acid for binding at the active site of SDH. In competitive inhibition K_m value increases, whereas V_{max} remains unchanged.

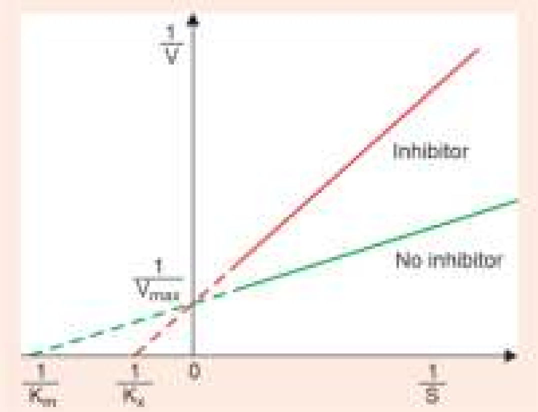


Fig. 2.5: Lineweaver plot of competitive inhibition

   B. *Non-competitive inhibition* (Fig. 2.6): The inhibitor has no structural resemblance with the substrate. The various non-competitive inhibitors are heavy metal ions such as Hg^+, Pb^{2+}, Ag^{2+}, etc. The non-competitive inhibitor binds at a site other than the active site on the enzyme surface. The inhibitor does not interfere with the enzyme-substrate binding. The catalysis is prevented probably due to a distortion in the enzyme conformation. In non-competitive inhibition, the K_m value is unchanged while V_{max} is lowered.

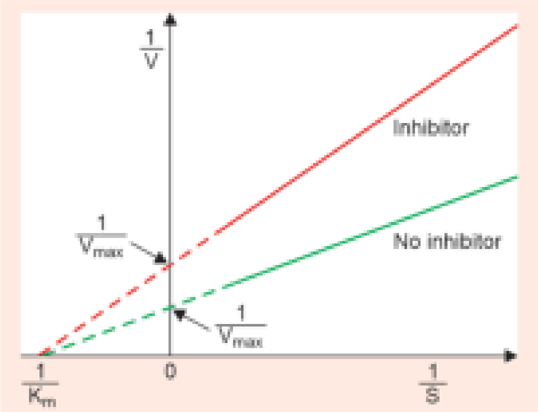


Fig. 2.6: Lineweaver plot of non-competitive inhibition

2. **Irreversible inhibition:** In irreversible inhibition, the inhibitors bind covalently with the enzymes and inactivate them. The inhibition of enzyme activity is irreversible.

   The examples of irreversible inhibition are as follows:

A. Iodoacetate is an irreversible inhibitor of the enzymes such as papain and glyceraldehyde 3-phosphate dehydrogenase. Iodoacetate combines with sulfhydryl (–SH) groups at the active site of these enzymes and make them inactive.

B. Diisopropyl fluorophosphate (DFP) irreversibly binds with enzymes containing serine, such as serine proteases, acetylcholine esterase, etc.

3. **Allosteric inhibition** (Fig. 2.7)**:** Some enzymes possess additional sites, known as allosteric sites, besides the active site. Certain substances referred to as allosteric modulators bind at the allosteric site and inhibit enzyme activity. The active site which binds the enzyme gets negatively modified; hence, the enzyme cannot react with the specific substrate. The allosteric modulators which inhibit enzyme activity are called negative allosteric modulators.

   Similarly, some allosteric modulators increase enzyme activity by positively modifying the active site by allosteric binding. These are called positive allosteric modifiers.

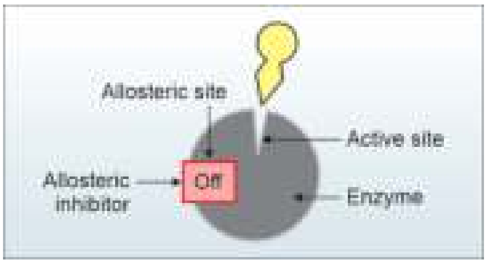


Fig. 2.7: Allosteric and active sites of an enzyme

Example

1. Rate of formation of citric acid in the citric acid cycle is controlled by the allosteric enzyme, fumarase.
2. Glucose 6-phosphatase is an allosteric activator of glycogen synthase (required in glycogenesis).

Enzyme Positive Modifiers

SAQ: What are inorganic positive modifiers of enzymes? Give four examples.

Ans: The activity of some enzymes can be reversibly increased by small inorganic molecules termed positive modifiers. Metal ions present in cells are known to be required for the full activity of one or more enzymes.

Examples:

$$Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}$$

In oxidoreductase activity, the metallic ions principally required are: Fe^{2+}, Mo^{2+}, and Cu^{2+}. For all the phosphate transfer reactions, Mg^{2+} ions are required. Sometimes anions such as Cl^- are also required to increase the activity of enzymes such as amylase.

BAQ: How do metallic ions accelerate enzyme-catalyzed reactions?

Ans: Metal may accelerate enzyme-catalyzed reactions by:

A. Direct participation in catalysis.

B. reacts with the substrate to form a true substrate.

C. Alters the equilibrium constant of the apparent overall reactions.

D. Brings about a conformational change in the protein by converting it from an inactive to an active conformation.

E. Combines first with the enzyme and forms a metalloenzyme (ME) which then binds with the substrate, and subsequently, a product is formed.

Enzyme Irreversible Inhibition

SAQ: Write on the use of irreversible inhibition of enzyme property in the treatment of alcohol addicts and the use of penicillin antibiotics.

Ans: Alcohol addicts can be treated with the drug Disulfiram. This drug irreversibly inhibits the enzyme alcohol dehydrogenase, leading to alcohol avoidance.

The antibiotic penicillin irreversibly inhibits serine-containing enzymes of bacteria, which are required for cell wall synthesis. In this process, bacteria that cause infection are killed.

SAQ: What is suicide inhibition?

Ans: "Suicide inhibition" is a type of irreversible inhibition, in which, the original inhibitor is converted to a more potent form to inhibit the activity of certain enzymes. For example, allopurinol drug (used to treat gout) is an inhibitor of xanthine oxidase. It acts on xanthine oxidase to form alloxanthine, which is a more potent inhibitor of xanthine oxidase.

Allosteric Inhibition of Enzymes

BAQ: What is the significance of allosteric inhibition in metabolic reactions? Give five examples.

Ans: Significance of allosteric inhibition:

1. The rate of formation of citric acid in the citric acid cycle is controlled by the allosteric enzyme, fumarase.
2. Glucose 6-phosphatase is an allosteric activator of glycogen synthase (required in glycogenesis).
3. Acetyl-CoA is an allosteric activator required in the synthesis of oxaloacetate (from pyruvate and bicarbonate) in gluconeogenesis (the reaction is catalyzed by pyruvate decarboxylase).
4. Acetyl-CoA also acts as an allosteric inhibitor of pyruvate decarboxylase.
5. The enzyme citrate synthase is an allosteric enzyme that exists in nearly all living cells and acts as a pace-making enzyme in the first step of the citric acid cycle (Krebs cycle).

THERAPEUTIC ENZYMES

SAQ: What are therapeutic enzymes? Give two examples.

Ans: Therapeutic enzymes are used to treat certain pathological conditions.

Examples

1. Pharmaceutical products in capsule form that contain digestive enzymes such as pepsin, and lipase to treat indigestion problems.
2. Streptokinase enzyme is used to dissolve blood clots formed in the blood vessels.

Competency achievement: The student should be able to:

BI2.5: Describe the clinical utility of various serum enzymes as markers of pathological conditions

LAQ: Describe the clinical utility of various serum enzymes as markers of pathological conditions.

Ans: Serum enzymes that do not perform any physiological function in serum or blood are known as non-functional enzymes. Their substrates are frequently absent from plasma. The enzymes are present in the blood of normal individuals at levels up to a millionfold lower than in tissues. These enzymes are: (a) The enzymes of exocrine secretions and (b) Intracellular enzymes. The nonfunctional enzymes enter the plasma in small amounts due to the continuous aging of cells or due to the diffusion through the damaged cell membrane. The low levels of non-functional enzymes found ordinarily in plasma arise apparently from the routine, normal destruction of erythrocytes, leukocytes, and other cells. Vigorous exercise also results in the release of small quantities of muscle enzymes. The plasma concentration of most enzymes remains fairly constant in the case of a normal individual. It will be altered if there is:

1. Change in the synthesis of enzymes within the cell, or
2. Change in the size of enzyme-forming tissue, or
3. Cellular damage, or
4. An alteration in the rate of inactivation and disposal of enzymes, or

5. An obstruction to a normal pathway of enzyme excretion

The enzymes of cellular metabolism are located within the tissue cells and are present there at high concentrations. Some exist freely in the cellular fluid, and others are contained in intracellular structures such as lysosomes and mitochondria. If the cell activity is impaired or damaged in some way, the cell membrane becomes permeable or ruptures. The cell contents, including their enzyme complements, are released into intracellular fluid and eventually reach the plasma. If a large volume of cells are affected, the plasma levels of these 'nonfunctional' enzymes increase suddenly. It has not been practical to assay enzymes in tissue on a routine basis. Instead, changes in enzyme concentrations in the blood can be studied by using photometric and spectrophotometric methods. The serum nonfunctional enzyme determinations may be helpful to:

1. Assess the severity of the organ damage
2. Follow the trend of the disease
3. Differentiate a particular type of disease, and
4. Determine post-operative risk.

Examples: Enzyme tests, affected organs, and related clinical conditions

Determination	**Diseased or affected organ/tissue**	**Condition**
a. Amylase	Pancreas	Acute pancreatitis
b. SGPT, SGOT	Liver	Infective hepatitis
c. CPK, SGOT, LDH	Heart	Myocardial infarction
d. Alkaline phosphatase	Bone	Rickets
e. Acid phosphatase	Prostate	Prostatic carcinoma
f. SGOT, LDH Lung Severe COVID-19		Pneumonia

SAQ: Name two enzymes that are elevated in liver disease.

Ans: Serum glutamate pyruvate transaminase (SGPT) and serum glutamate aspartate transaminase (SGOT).

SAQ: Name two enzymes that are elevated in a heart attack.

Ans: Creatine kinase (CK) and serum glutamate aspartate transaminase (SGOT).

SAQ: Name two enzymes that are elevated in severe lung disease (e.g. COVID-19).

Ans: Serum glutamate aspartate transaminase (SGOT) and lactate dehydrogenase (LDH).

SAQ: Describe COVID-19 under the following heads:

1. Cause of the disease
2. Names of elevated serum enzymes with reason
3. The first line of treatment
4. Preventive measure

Ans:

1. SARS-CoV-2 virus infection.
2. SGOT and LDH, due to inflammation of the lungs.
3. Supportive treatment with an appropriate dose of paracetamol to decrease body temperature, fluid replacement (IV saline), and if necessary, (if blood oxygen saturation drops below 90%) appropriate oxygen supply.
4. Vaccination with COVAXIN or COVISHIELD against COVID-19, with COVID-19 safety protocol.

SAQ: Describe viral hepatitis under the following heads:

1. Cause of the disease
2. Names of elevated serum enzymes with reason
3. The first line of treatment
4. Preventive measure

Ans:

1. Mainly hepatitis A, or hepatitis B infection.
2. Very high SGPT and SGOT due to inflammation of the liver.

3. Supportive treatment with food restrictions, adequate carbohydrates, reduced lipids, and adequate fluid intake and rest.
4. Safe food and water habits (hepatitis A), vaccination against hepatitis B.

SAQ: Write briefly on the use of enzymes in the reagents used for the determination of various components of body fluids.

Ans: Enzymes are used as an important part of reagent kits used in the determination of body fluid (serum, plasma, urine, CSF. ETC) components like glucose, urea, uric acid, cholesterol, etc. For example, glucose reagent contains the enzymes, glucose oxidase and peroxidase. Urea reagent contains the enzyme, urease. The uric acid reagent contains uricase and the cholesterol reagent contains cholesterol oxidase. These enzymatic kits are very easy to use in mono-step methods, that require a very short time for the completion of the test procedures.

Multiple Choice Questions

Q1. Which of the following factors affect enzyme activity?

A. Buffers B. pH
C. Temperature D. A, B, and C

Q2. Following myocardial infarction, which enzyme rises during 1–3 days?

A. SGOT B. CPK
C. SHBD D. LDH

Q3. Serum acid phosphatase level increases in

A. Liver diseases
B. Acute pancreatitis
C. Renal diseases
D. Metastatic carcinoma of the prostate

Q4. Serum alkaline phosphatase level increases in

A. Rickets
B. Myocardial infarction
C. Posthepatic condition
D. Both A and C

Q5. Serum amylase level increases in

A. Acute pancreatitis
B. Renal disease
C. Hepatitis
D. B and C

Q6. Following myocardial infarction, the enzymes elevated up to 3 weeks are

A. SGOT B. Total CK
C. SHBD D. A and B

Q7. LD1 is elevated in

A. Myocardial infarction
B. Kidney disease
C. Liver disease
D. Prehepatic condition

Q8. The CK isoenzymes present in cardiac muscle are of type

A. MB B. MM
C. BB D. None of these

Q9. An example of a functional plasma enzyme is

A. CPK B. Amylase
C. SGOT D. Lipoprotein lipase

Q10. Which of the following are nonfunctional plasma enzymes?

A. Lipoprotein lipase
B. SGPT
C. Serum alkaline phosphatase
D. B and C

Q11. The optimum pH of serum amylase is

A. 10.3 B. 2.0
C. 7.0 D. 4.5

Q12. The substrate for amylase is

A. Starch B. Glucose
C. Lactose D. Ribose

Q13. Which of these ions activate salivary amylase activity

A. Calcium B. Chloride
C. Sodium D. Potassium

Q14. The pancreatic amylase activity can be increased in the presence of

A. Magnesium ions
B. Bile salts
C. Hydrochloric acid
D. Chloride ions

Q15. Coenzymes combine with

A. Apoenzymes B. Holoenzymes
C. Metallic ions D. Proenzymes

Q16. Coenzymes are required in which of the following reactions?

A. Oxidation–reduction
B. Transamination
C. Phosphorylation
D. A, B, and C

Q17. Which of the following coenzymes takes part in hydrogen transfer reactions?
A. Coenzyme Q
B. Tetrahydrofolate
C. Coenzyme A7
D. ATP

Q18. In the conversion of glucose to glucose 6-phosphate, the coenzyme is
A. ATP B. ADP
C. NAD D. Coenzyme A

Q19. Which of the following is a coenzyme required in transamination reactions?
A. Pyridoxal phosphate
B. Coenzyme Q
C. Coenzyme A
D. NAD

Q20. Coenzyme A contains which of the following vitamins?
A. Folic acid
B. Pantothenic acid
C. Vitamin C
D. Niacinamide

Q21. Cobamides contain which of the following vitamins?
A. Pantothenic acid
B. Vitamin B_{12}
C. Folic acid
D. Riboflavin

Q22. A coenzyme required in carboxylation reactions is
A. Coenzyme Q B. Lipoic acid
C. Biotin D. NAD

Q23. Which of the following coenzymes takes part in tissue respiration?
A. Coenzyme A B. NAD
C. Coenzyme Q D. ATP

Q24. The enzyme hexokinase is a
A. Ligase
B. Oxidoreductase
C. Lyase
D. Transferase

Q25. Which of the following is a hydrolase?
A. Trypsin B. SGPT
C. CK D. LDH

Q26. Enzymes that catalyze the binding of two substrates are
A. Ligases B. Hydrolases
C. Transferases D. Lyases

Q27. Allosteric inhibition is also a
A. Non-competitive inhibition
B. Feedback inhibition
C. Competitive inhibition
D. B or C

Q28. An allosteric enzyme is generally inhibited by
A. Last substrate concentration
B. Initial substrate of the pathway
C. Product of the pathway
D. Metallic ions

Q29. When the velocity of an enzymatic reaction equals V_{max}, the substrate concentration is:
A. Far above the K_m
B. Equal to K_m
C. Half of K_m
D. 1/4th of K_m

Q30. In the Lineweaver-Burk plot, the Y-intercept represents
A. V_{max} B. $1/K_m$
C. K_m D. A and B

Q31. In competitive inhibition, the inhibitor
A. Irreversibly binds with the enzyme
B. Competes with the enzyme
C. Competes with the substrate
D. Binds with the substrate

Q32. Competitive inhibitors
A. Increase the V_{max}
B. Increase the K_m
C. Decrease the K_m
D. Decrease the V_{max}

Q33. Competitive inhibition can be relieved by raising the
A. Enzyme concentration
B. Substrate concentration
C. Ionic concentration of buffer
D. A and C

Q34. An inorganic ion required for the full activity of an enzyme is known as
A. Positive modifier
B. Negative modifier
C. Coenzyme
D. B and C

Q35. All of the following are iron-containing enzymes *except*
A. Cytochrome oxidase
B. Peroxidase
C. Catalase
D. Carbonic anhydrase

Q36. This reaction is catalyzed by: Alanine + alpha-keto-glutarate $\rightleftharpoons$ Glutamic acid + Pyruvate

A. Alkaline phosphatase
B. SGPT
C. SGOT
D. LDH

Q37. This reaction is catalyzed by: Lactate + NAD $\rightleftharpoons$ Pyruvate + NADH

A. LDH B. MDH
C. SGPT D. SGOT

Q38. The Michaelis-Menten hypothesis

A. States that the reaction rate is proportional to enzyme concentration
B. States that the rate of an enzyme-catalyzed reaction may be independent of substrate concentration
C. Assumes the formation of an enzyme-substrate complex
D. Both B and C

Q39. Diastase acts on

A. Starch B. Maltose
C. Cellulose D. Sucrose

Q40. Allosteric enzymes contain

A. One subunit
B. Many subunits
C. Two peptide chains
D. Four peptide chains

Q41. Feedback inhibition of enzyme action is affected by

A. the pH of the substrate
B. Substrate concentration
C. A and B
D. End product

Q42. Template lock and key theory of enzyme action is outdated since

A. It indicates the fixed fitting size of enzyme and substrate molecules
B. It indicates enzyme concentration speeds up the reaction
C. It indicates substrate concentration speeds up the reaction
D. Coenzymes are required to speed up certain reactions

Q43. Site of enzyme synthesis in a cell is

A. Golgi bodies
B. Rough endoplasmic reticulum
C. Mitochondria
D. All of these

Q44. Out of the total enzymes present in a cell, a mitochondrion has average

A. about 70% enzymes
B. about 10% enzymes
C. about 20% enzymes
D. about 5% enzymes

Q45. Which one of the following is a coenzyme?

A. Ascorbic acid
B. Retinol
C. Pyridoxal phosphate
D. Cholecalciferol

Q46. In the cell, digestive enzymes are found mainly in

A. Golgi apparatus B. Lysosomes
C. Ribosomes D. Mitochondria

Q47. About 17 patients were admitted to the emergency ward with a history of drinking illicit (illegal) alcohol (average consumption 150–250 ml). They were immediately treated by giving them each about 200 ml of ethyl alcohol. The action of ethyl alcohol was:

A. As a competitive inhibitor to methyl alcohol
B. As a fluid replacement
C. As an allosteric inhibitor of methyl alcohol
D. All of the above

Q48. An 18-year-old student suffered from loss of appetite, nausea, mild fever, and pain in the right hypochondrium. On examination, it was found that the liver was palpable and tender. Which serum enzyme determination will indicate liver disease?

A. CK B. SGPT
C. SCOT D. Both B and C

Q49. A 55-year-old female tested positive for COVID-19 disease. Her blood tests indicated normal values of SGPT and very high values of SGOT and LDH. The blood test report indicates

A. Severe Heart disease
B. Severe liver disease
C. Severe lung disease
D. A, B, and C

Q50. A 46-year-old man presented with symptoms of a heart attack. Which serum enzyme determinations will confirm heart disease?

A. CK B. SGPT
C. SGOT D. Both A and C

Answers

1. D	**2.** B	**3.** D	**4.** D	**5.** A	**6.** D	**7.** A	**8.** A	**9.** D	**10.** D
11. C	**12.** A	**13.** B	**14.** D	**15.** A	**16.** D	**17.** A	**18.** A	**19.** A	**20.** B
21. B	**22.** D	**23.** C	**24.** D	**25.** A	**26.** A	**27.** C	**28.** C	**29.** A	**30.** B
31. C	**32.** B	**33.** B	**34.** A	**35.** D	**36.** B	**37.** A	**38.** C	**39.** A	**40.** B
41. D	**42.** A	**43.** B	**44.** A	**45.** C	**46.** B				

Explanations are given wherever necessary

47. A: Explanation: Ethyl alcohol is a competitive inhibitor of methyl alcohol and reduces the toxic effects of methyl alcohol

48. D: Explanation: Since concentrations of SGPT and SGOT are very high in liver tissue, both are elevated in liver disease.

49. C: Explanation: Since lung tissue is rich in SGOT and LDH, coronavirus predominantly infects lungs. Normal SGPT indicates that probably liver was not affected. There were no symptoms of myocardial infarction.

50. D: Explanation: Both CK and SGOT (and not SGPT) are elevated following a heart attack.

Case Studies

Case 1: An 18-year-old student suffered from loss of appetite, nausea, mild fever, and pain in the right hypochondrium. On examination, it was found that the liver was palpable and tender. His laboratory test reports were as follows:

	Normal values
SGPT: 650 IU	5–35 IU
SGOT: 335 IU	8–40 IU
Alkaline phosphatase: 95 IU	20–80 IU

1. What is the probable diagnosis?

Ans: Liver disease (hepatic condition), since both SGPT and SGOT are increased and serum alkaline phosphatase is only marginally increased.

2. What is the mechanism behind the increase in SGPT and SGOT?

Ans: It may be due to microbial infection leading to inflammation and necrosis of hepatic cells. It is necessary to find out from case history if liver cell damage is related to specific drugs.

3. What additional tests are required to confirm the diagnosis?

Ans: Routine urine examination and serum bilirubin determination to confirm jaundice and hepatic condition.

4. What is the probable line of treatment?

Ans: If it is viral infective hepatitis, usually the patient gets well after 3 weeks. Bacterial infection requires appropriate antibiotic treatment. If the hepatic condition is due to drug or organic solvent intake, stoppage of drugs, or intake of organic solvent.

5. Show horizontal integration of symptoms and test reports of this patient with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Affected liver

Horizontal integration with physiology

Disturbed bile pigment and bile salt excretion by the liver

Vertical integration with general medicine

Study of jaundice

Vertical integration with medical microbiology

Study of microorganisms that may have infected liver

Case 2: A 54-year-old man presented with jaundice. There was no history of injections or transfusions. He did not drink alcohol. He complained of intense pruritus during the past 2–3 months. His laboratory test reports were as follows:

	Normal range
SGPT: 150 IU	5–35 IU
SGOT: 180 IU	8–40 IU
Alkaline phosphatase: 358 IU	20–80 IU

1. What is the probable diagnosis?

Ans: Post-hepatic condition, since both SGPT and SGOT are only moderately increased and serum alkaline phosphatase levels are very high.

2. What is the mechanism behind the very high increase in alkaline phosphatase and moderate increases in SGPT and SGOT?

Ans: Serum alkaline phosphatase values increase when there is an obstruction to the flow of bile (post-hepatic condition) and the liver is not significantly affected. Hence, SGPT and SGOT are only moderately increased.

3. What additional tests are required to confirm the diagnosis?

Ans: Routine urine examination and serum bilirubin determination to confirm jaundice and post-hepatic condition.

4. What is the probable line of treatment?

Ans: Surgical removal of the obstructing component to the flow of bile.

5. Show horizontal integration of symptoms and test reports of this patient with anatomy, physiology and nutrition.

Ans: Horizontal integration with anatomy
Affected gall bladder

Horizontal integration with physiology
Disturbed bile pigment and bile salt excretion by the liver

Vertical integration with general medicine
Study of jaundice

Vertical integration with surgical medicine
Removal of obstructing components to the flow of bile in the intestine by surgical methods.

Vertical integration with pathology
Study of cause, pathophysiology and prognosis of hepatitis

Case 3: A 72-year-old man experienced a sensation of not emptying his bladder after finishing urinating. He also experienced a weak urinary stream often while urinating. His routine urine examination report indicated normal physical, chemical, and microscopic observations. Following were additional blood test reports:

	Normal range
Serum total acid phosphatase: 21 IU	0.9–12 IU
Serum prostatic acid phosphatase: 14 IU	0.0–4.0 IU

1. What is the probable diagnosis?

Ans: Enlarged prostate gland, since prostatic acid phosphatase level is very high.

2. What is the mechanism behind the increase in prostate size?

Ans: It may be due to the advanced age of the patient or due to prostate carcinoma.

3. What additional tests are required to confirm the diagnosis?

Ans: Histopathology studies to find out if prostatic enlargement is due to cancer or if it is benign. Similarly, it is necessary to find out levels of blood urea nitrogen and serum creatinine to find out the status of the pre-renal condition.

4. What is the probable line of treatment?

Ans: Alpha blockers could be prescribed as the first line of treatment, which relax bladder neck muscles and muscle fibers in the prostate, facilitating easier urination. However, if the size of the prostate is significantly increased, then surgical removal of the enlarged portion of the prostate is considered. Prostatic cancers are treated by chemotherapy, radiotherapy, and immunotherapy.

5. Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology.

Ans: Horizontal integration with anatomy

Affected kidneys due to enlargement of the prostate gland.

Horizontal integration with physiology

Obstruction to the excretion of urine due to enlargement of the prostate gland.

Vertical integration with surgical medicine

Consideration of surgical removal of the prostate gland.

Vertical integration with pathology

Study of cause, pathophysiology and prognosis of post-hepatic condition.

Case 4: A 65-year-old woman complained of severe constant epigastric pain radiating to the back. Following were additional tests performed apart from the usual body profile tests:

	Normal range
Serum amylase: 560 IU	30–110 IU
Urine (random) amylase: 755 IU	32–640 IU (24-hour specimen)
Serum lipase: 310 IU	Up to 200 IU

1. What is the probable diagnosis?

Ans: Acute pancreatitis, since serum amylase and lipase enzymes are increased and the patient suffered from severe epigastric pain.

2. What is the mechanism behind the increase in serum amylase and lipase?

Ans: Inflammation of the pancreas leads to an increase in serum amylase and lipase.

3. What are the possible reasons for acute pancreatitis?

Ans: Excessive alcohol intake, formation of gall stones, viral infection, pancreatic cancer, cystic fibrosis, very high levels of serum triglycerides, or reason may be unknown.

4. What is the probable line of treatment?

Ans: It depends on the exact cause that leads to acute pancreatitis. Viral infections may resolve in two weeks, while bacterial infections could be treated with appropriate antibiotics.

5. Show horizontal integration of symptoms and test reports of this patient with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Affected pancreas

Horizontal integration with physiology

Affected excretion of pancreatic juice in the intestine

Horizontal integration with general medicine

Study of causes of acute pancreatitis

Vertical integration with pathology

Study of cause, pathophysiology and prognosis of prostatic enlargement

Chapter 3

Biologic Oxidation, Respiratory Chain, Lipid Peroxidation, Antioxidants

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each

BIOLOGICAL OXIDATION

Biological oxidation means energy-producing reactions in living cells involving the transfer of hydrogen atoms or electrons from one molecule to another. Following are some redox systems involved in mammalian oxidation-reduction reactions:

$H^+/2H$, pyruvate/lactate, NAD/NADH, acetoacetate/3-hydroxybutyrate, fumarate/succinate, oxaloacetate/malate, cytochrome b; Fe^{+++}/Fe^{++}, cytochrome c; Fe^{+++}/Fe^{++}, oxygen/water, etc. These redox systems form a chain and carry electrons from the metabolites to oxygen. The ultimate electron acceptor in the aerobic cell is oxygen. It has an avidity for electrons. When it accepts electrons, protons from the medium join to produce water.

$$O_2 + 4e + 4H^+ \rightarrow 2H_2O$$

The first electron carrier involved in the oxidation of most metabolites is nicotinamide adenine dinucleotide (NAD^+). Nicotinamide group in NAD forms from vitamin niacin (nicotinic acid). Similarly, another electron carrier is flavin adenine dinucleotide (FAD). It is derived from vitamin B_2 (riboflavin).

SAQ: Mention four redox systems involved in biological oxidation–reduction reactions.

Ans: $H^+/2H$, pyruvate/lactate, NAD/NADH, and fumarate/ succinate.

THE RESPIRATORY CHAIN (Fig. 3.1)

BAQ: Describe the respiratory chain.

Ans: End products of digestion and absorption are glucose (from carbohydrates), amino acids (from proteins), fatty acids, and glycerol (from lipids) are ultimately metabolized to Acetyl-CoA, which passes through a citric acid cycle to form reducing equivalents (in the form of H^+ ions). These reducing equivalents are passed through the respiratory chain in the mitochondria to generate ATP molecules.

There are four (I, II, III, IV) respiratory chain complexes. Each of these complexes acts as a proton pump. The inner membrane of these complexes is impermeable to small molecules.

Complex I (NADH-Q oxidoreductase): In this complex electrons are transferred from NADH to coenzyme Q.

Complex II (Succinate Q reductase): It has more positive redox potential and passes electrons to coenzyme Q.

Complex III (Q-cytochrome c oxidoreductase): It passes the electrons on to cytochrome c.

Complex IV (Cytochrome c oxidase): It completes the chain, passing the electrons to O_2 and causing it to be reduced to H_2O.

Complex V (ATP synthase): The proton motive force drives a membrane-located ATP synthase that in the presence of Pi and ADP forms ATP. ATP synthase is embedded in the inner membrane, together with the respiratory chain complexes.

Protons that are pumped by the proton pump accumulate outside the inner membrane creating an electrochemical potential difference across the membranes which consist of a difference in chemical potential (pH) and an electrical potential. This electrical difference is then used to drive a membrane located in ATP synthase (complex V) which in the presence of Pi and ADP forms ATP molecules. ATP is the universal chemical energy currency of life. ATP is consumed by the cells to drive cellular processes that require energy such as synthesis of macromolecules, active transport of molecules across the membrane, etc.

STAGES IN THE PRODUCTION OF ENERGY

Competency achievement: The student should be able to:

BI6.6: Describe the biochemical processes involved in the generation of energy in cells

BAQ: Describe the biochemical processes involved in the generation of energy in cells.

Ans: The energy is generated through the following stages:

Stage 1: Anaerobic glycolysis

In this stage, oxygen is not involved (since it may be inadequate in the muscles) and two ATP molecules are produced per one molecule of lysed glucose. NAD is required as a coenzyme and the end products are pyruvate and NADH. The rate of glycolysis is dramatically increased in the initial phase of emergency flight reactions to produce muscular activities. Since the initial concentration of NAD obtained from mitochondria is low, pyruvic acid is converted to lactic acid in the presence of the enzyme lactate dehydrogenase, as shown in the following reaction to produce an adequate amount of NAD molecules in emergencies:

$$\text{Pyruvate} + \text{NADH}^- \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

Lactate is converted back to glucose in the liver. However, muscular and joint pain observed after excessive exercise is due to the accumulation of lactic acid in muscles and joints. Thus, the objective of anaerobic glycolysis is to re-oxidize NADH and permit continuous ATP production required for muscular activities.

In aerobic glycolysis in the presence of oxygen, NADH is re-oxidized to NAD in the mitochondria, and pyruvate is oxidized to CO_2 and water.

Stage 2: The citric acid cycle

Pyruvic acid produced in the glycolysis is converted to acetyl-CoA, which enters the citric acid cycle, and at the end of the citric acid cycle, 2 molecules of carbon dioxide, 3 molecules of NADH, one molecule of $FADH_2$, one molecule of GTP are formed.

Mitochondria are the main energy-generating units of aerobic cells where most of the ATP molecules are produced. The inner membrane of mitochondria contains a specific transport system and it is increased by several folds called cristae. A transport system takes pyruvic acid formed in stage 1 into the mitochondria. Pyruvic acid is then converted to three molecules of CO_2, NADH is converted to NAD, and FADH to FAD.

Stage 3: Electron transfer through the electron transport chain. An electron transport chain (ETC) couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O_2) with the transfer of H^+ ions (protons) across the mitochondrial membrane.

The electrical difference is then used to drive a membrane located in ATP synthase (complex V) which in the presence of inorganic phosphorus and ADP forms ATP molecules. ATP is the universal chemical energy currency of life. ATP is consumed by the cells to drive cellular processes that require energy such as synthesis of macromolecules, active transport of molecules across the membrane, etc.

THE PATHWAY OF ELECTRONS (BASED ON THE CHEMIOSMOTIC HYPOTHESIS)

BAQ: Describe the pathway of electrons (based on the chemiosmotic hypothesis).

Ans: Chemiosmosis is the movement of ions across a selectively permeable membrane, down their electrochemical gradient. More specifically, it relates to the generation of ATP by the movement of hydrogen ions across a membrane during cellular respiration. Electron transfer in mitochondria is accompanied by an asymmetric release of protons on one side of the inner membrane of mitochondria.

The following four (I, II, III, IV) respiratory chain complexes are present on the inner membrane of mitochondria. Each of these complexes acts as a proton pump. The inner membrane of these complexes is impermeable to small molecules.

The pathway of electrons occurs as follows (Fig. 3.1):

Complex I (NADH-Q oxidoreductase): In this complex electrons are transferred from NADH to coenzyme Q.

Complex II (succinate Q reductase): It has more positive redox potential and passes electrons to coenzyme Q.

Complex III (Q-cytochrome c oxidoreductase): It passes the electrons on to cytochrome c.

Complex IV (cytochrome c oxidase): It completes the chain, passing the electrons to O_2 and causing it to be reduced to H_2O.

Complex V (ATP synthase): The proton motive force drives a membrane-located ATP synthase that in the presence of Pi and ADP forms ATP. ATP synthase is embedded in the inner membrane, together with the respiratory chain complexes.

ATP is the universal chemical energy currency of life. ATP is consumed by the cells to drive cellular processes that require energy such as synthesis of macromolecules, active transport of molecules across the membrane, etc.

The overall electron transport chain is as follows:

NADH → Complex I → Q → Complex III → cytochrome c → Complex IV → O_2

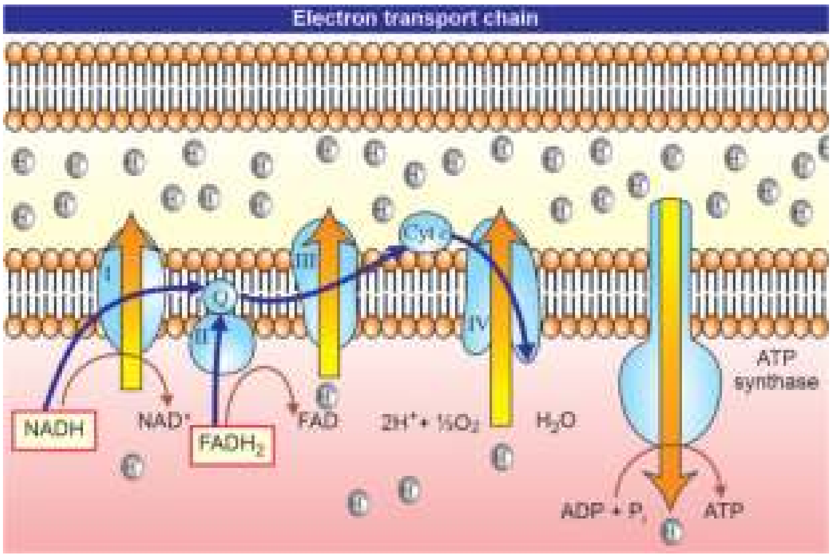


Fig. 3.1: Electron transfer chain (respiratory chain)

Oxidative Phosphorylation

SAQ: What is oxidative phosphorylation?

Ans: The process by which electrons are transferred from NADH or FADH2 to O_2 by a series of electron transfer carriers, in the mitochondrial inner membrane, is known as oxidative phosphorylation. It is through this process that ATP molecules form as a result of the transfer of electrons.

For oxidative phosphorylation to proceed, the following two conditions are required:

1. The inner mitochondrial membrane must be physically intact so that the protons can enter through it.
2. A high concentration of protons must be developed on the outside of the inner membrane.

SAQ: Enumerate the names of 4 enzymes that play important roles in the antioxidant defense systems of the body.

Ans: Catalase, Superoxidase dismutase, peroxidases, and dehydrogenases.

The Antioxidant Defence Systems of the Body

Competency achievement: The student should be able to:

BI7.6: Describe the antioxidant defense systems of the body

LAQ: Describe the antioxidant defense systems of the body.

Ans: The following enzyme systems play a very important role in neutralizing the harmful effects of oxidants produced in the body at the cellular level:

Role of enzymes

Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. They form water or hydrogen peroxide at the end of the reaction.

Cytochrome oxidase is a hemoprotein present in many tissues including myoglobin and cytochromes. It is a terminal component of the chain of respiratory carriers found in mitochondria and transfers electrons resulting from the oxidation of substrate molecules by dehydrogenases to oxygen.

Flavoprotein enzymes are also oxidases that contain flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) as prosthetic groups. The flavoproteins mediate the transfer of electrons from a series of mitochondrial flavoenzymes to the respiratory chain.

Dehydrogenases transfer hydrogen from one substrate to another substrate in a coupled oxidation–reduction reaction.

Peroxidases are found in leukocytes, platelets, milk, and other tissues. The prosthetic (tightly bound cofactor) group in peroxidases is protoheme. Hydrogen peroxide produced in metabolic reactions is a toxic component. It is reduced to water by the catalytic action of peroxidases that involve several substances, which act as electron acceptors.

Catalase enzyme is found in blood, kidney, bone marrow, liver, mucous membrane, etc. It is a hemoprotein, which contains four heme groups. Catalase is capable of using one molecule of hydrogen peroxide as an electron donor and another molecule as an electron acceptor. Mainly, it acts on hydrogen peroxide to produce water and oxygen.

Superoxide dismutase is found in all major aerobic tissues in the mitochondria and the cytosol. It acts as both an oxidant and reductant. In metabolic reactions, the transfer of a single electron to oxygen generates the formation of potentially damaging superoxide radicals. The destructive effects of these radicals are amplified by free-radical chain reactions. Superoxide dismutase acts on superoxide molecules and the formation of hydrogen peroxide takes place, which is converted to water by the catalytic action of catalases.

Cytochrome 450 is an important heme-containing monooxygenase. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes, which in turn are oxidized by substrates in a series of enzymatic reactions known as the hydroxylase cycle.

SUBSTANCES THAT INHIBIT THE RESPIRATORY CHAIN

BAQ: Enumerate the names of substances that inhibit the respiratory chain and their respective actions.

Ans: The following substances inhibit the respiratory chain and their respective actions:

- Barbiturates inhibit electron transfer via complex I by blocking the transfer from Fe-S to Q.
- Antimycin A and dimercaprol inhibit the respiratory chain at complex III.
- Cyanide, carbon monoxide, and sulfur dioxide inhibit complex IV and can arrest respiration.
- Malonate is a competitive inhibitor of complex II.
- Oligomycin completely blocks oxidation and phosphorylation by blocking the flow of protons through ATP synthase.
- Azide inhibits electron transport from complex IV.
- 2, 4 dinitrophenol acts as an uncoupling agent at the transmembrane H^+ carrier.
- Pentachlorophenol acts as an uncoupling agent at the transmembrane H^+ carrier.

REACTIVE OXYGEN SPECIES AND SUPEROXIDE IONS

Competency achievement: The student should be able to:

BI7.7: Describe the role of oxidative stress in the pathogenesis of conditions such as cancer, complications of diabetes mellitus and atherosclerosis

BAQ: What are reactive oxygen species, superoxide ions, and their harmful effects on body systems?

Ans: Reactive oxygen species (ROS) are highly reactive chemicals formed from diatomic oxygen. The use of oxygen as part of the process for generating metabolic energy in the form of ATP molecules produces reactive oxygen species. In this process, the superoxide anion is produced as a by-product of several steps in the electron transport chain. The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (–OH–) and the superoxide anion (O_2^-).

ROS can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Lipid peroxidation is the process in which free radicals capture electrons from the lipids in cell membranes, resulting in cell damage. DNA damage can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation, and protein degradation.

Superoxide ions are formed by the combination of one molecule of dioxygen (O_2) and one electron (e^-). Superoxide is a compound that possesses the superoxide anion with the chemical formula O_2^-. With one unpaired electron, the superoxide ion is a free radical. Superoxide is biologically quite toxic and is used by the immune system to kill invading microorganisms. In phagocytes, superoxide is produced in large quantities by the enzyme NADPH oxidase for use in oxygen-dependent killing mechanisms of invading pathogens.

When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress, that can significantly alter the cell membranes and other structures such as DNA, proteins, lipids, etc. Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed, as seen in immunocompromised persons. In other words, oxidative stress results from an imbalance between the formation and neutralization of ROS.

The oxidative stress can also trigger the inflammatory response, which, in turn, produces more free radicals that can lead to further oxidative stress, creating a vicious cycle. Chronic inflammation due to oxidative stress may lead to several conditions, including cancer, diabetes, autoimmune disorders, cardiovascular disease, and arthritis.

The human body has several mechanisms to counteract oxidative stress by producing various enzymes such as catalase, superoxide dismutase, peroxidase, glutathione, and melatonin, which are capable of destroying oxidants. Vitamins such as vitamins C and E; supplied through foods and supplements also act like antioxidants.

LIPID PEROXIDATION

BAQ: Write a note on lipid peroxidation.

Ans: Lipid peroxidation is the process in which free radicals capture electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. Most often it affects polyunsaturated fatty acids present in the cells since they contain multiple double bonds and in between these bonds are methylene $–CH_2$ groups that possess especially reactive hydrogens. The lipid peroxidation reaction consists of three major steps: Initiation, propagation, and termination.

Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH and H_2O_2, which combines with a hydrogen atom to make water and a fatty acid radical.

During the propagation stage, when a radical reacts with a nonradical component, it produces another radical. Hence the process is called a “chain reaction mechanism.”

In the termination stage, the radical reaction stops when two radicals react and produce a non-radical species. This takes place only when the concentration of radical species is sufficiently high.

Living organisms have evolved different molecules that speed up termination by catching free radicals and, therefore, protecting the cell membrane. One such important antioxidant is vitamin E. Other antioxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

If lipid peroxidation is not terminated, there will be damage to the cell membrane, which consists mainly of lipids. In addition, end products of lipid peroxidation may be mutagenic and carcinogenic.

IMPORTANCE OF ANTIOXIDANT LEVELS IN FOOD

BAQ: Write a note on the importance of antioxidants in food.

Ans: Free radicals produced in the body due to cigarette smoke, drugs, pollution, and stress related to sickness are responsible for causing various degenerative diseases such as cancer, diabetes mellitus, cataract, cardiovascular diseases, the decline in immune system functions, brain dysfunction, etc. Many interventional studies show the beneficial benefits of adequate antioxidants through fresh fruits, vegetables, nuts, grains, and plant oils. These foods supply adequate antioxidants in the form of vitamin C, vitamin E, beta-carotene, and lipoic acid, which have protective effects by decreasing oxidative damage to various cells, DNA, and various organs.

Antioxidants are commonly used as medications to treat various forms of brain injury. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (motor neuron disease).

Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes, and nuts. Some antioxidants such as lycopene and ascorbic acid can be destroyed by long-term storage or prolonged cooking. Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole wheat cereals and tea. In general, processed foods contain fewer antioxidants than fresh and uncooked foods, since the preparation processes may expose the food to oxygen.

BAQ: Why antioxidants are added as food additives? Name two antioxidant food additives.

Ans: Antioxidants are used as food additives to prevent supplement food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food. Hence, food is preserved by mixing with food additives, keeping in the dark, sealing it in containers, and coating it in wax. The names of antioxidant food additives are: Tocopherols and calcium ascorbate.

Multiple Choice Questions

Q1. The location of the electron transport system

A. In the matrix of the mitochondrion
B. On the outer membrane of the mitochondrion
C. On the cristae of the mitochondrion
D. In the cytoplasm

Q2. The ETS receives electrons from

A. FAD
B. $FADH_2$
C. NAD^+
D. ADP

Q3. When a component of the ETS receives electrons, it is

A. Neutralized
B. Hydrolyzed
C. Reduced
D. Oxidized

Q4. The mitochondrion is surrounded by

A. 2 membranes
B. 1 membrane
C. 4 membranes
D. 3 membranes

Q5. As electrons travel down the ETS, they release

A. ADP
B. Energy
C. ATP
D. Hydrogen ions

Q6. The energy released by electrons moving down the ETS causes the pumping of

A. ADP
B. H^+ ions
C. Water
D. NAD^+

Q7. Hydrogen ions are pumped from

A. Matrix; to the cytoplasm
B. Matrix; to the intermembrane space
C. Cytoplasm; to the matrix
D. Cytoplasm; to the intermembrane space

Q8. H^+ moves down its concentration gradient into the matrix by

A. Simple diffusion
B. Facilitated diffusion
C. Osmosis
D. Active transport

Q9. When the H^+ re-enter the matrix, they activate the enzyme

A. Cytochrome oxidase
B. ATP synthase
C. Protease
D. Lipoprotein lipase

Q10. The final electron acceptor is

A. NAD^+
B. Carbon dioxide
C. Oxygen
D. FAD

Q11. When electrons are transferred to oxygen at the end of the ETS, they also unite with H^+ to form

A. Water
B. Glucose
C. Carbon dioxide
D. ADP

Q12. Cyanide is a metabolic poison that stops the ETS by directly blocking the actions of

A. NAD^+
B. Cytochrome oxidase
C. ATP synthase
D. NADH

Q13. Each NADH entering the ETS from Krebs cycle produces

A. 1 ATP
B. 2 ATP
C. 4 ATP
D. 3 ATP

Q14. Each $FADH_2$ entering the ETS from Krebs cycle produces

A. 1 ATP
B. 3 ATP
C. 2 ATP
D. 4 ATP

Q15. Oxygen is needed in the ETS to

A. Prevent a pile-up of electrons
B. Prevent a pile-up of NAD^+
C. Prevent a pile-up of hydrogen ions
D. Prevent a pile-up of ADP

Q16. Electron-transfer reactions in mitochondria, almost all of the oxygen in breathing is converted to

A. Carbon dioxide
B. Acetyl-CoA
C. Water
D. None of the above

Q17. Antimycin A and dimercaprol inhibit the respiratory chain at

A. Complex I
B. Complex III
C. Complex II
D. Complex IV

Q18. In normal mitochondria, the rate of oxidation (NADH consumption) will

A. Decrease when cyanide is used to prevent electron transfer through the cytochrome a + a3 complex.
B. Be very low if the ATP synthase is inhibited, but increase when an uncoupler is added.
C. Decrease if mitochondrial ADP is depleted.
D. All of the above are true.

Q19. Which of the following statements about the chemiosmotic theory is correct?

A. It predicts that oxidative phosphorylation can occur even in the absence of an intact inner mitochondrial membrane.
B. Electron transfer in mitochondria is accompanied by the movement of protons across the mitochondrial membrane, down their electrochemical gradient.
C. Electron transfer in mitochondria is accompanied by an exchange of sodium and potassium ions.
D. The membrane cytochrome oxidase is required for chemiosmosis.

Q20. During oxidative phosphorylation, the proton motive force that is generated by electron transport is used to

A. Generate the substrates (ADP and Pi) for the ATP synthase.
B. Induce a conformational change in the ATP synthase.
C. Oxidize NADH to NAD^+
D. Reduce O_2 to H_2O

Q21. Barbiturates inhibit electron chain by inhibiting

A. Complex I
B. Complex II
C. Complex III
D. Complex IV

Q22. Carbon monoxide inhibits electron chain by inhibiting

A. Complex I
B. Complex II
C. Complex III
D. Complex IV

Q23. All these reactions take place in mitochondria, *except*

A. EM pathway
B. Urea cycle
C. Krebs cycle
D. Electron transfer

Q24. Living cells require which of the following as a component of NAD, ATP, and flavin nucleotides?

A. Sulfur
B. Inorganic phosphorus
C. Magnesium ions
D. Ferrous ions

Q25. Detoxication of drugs is performed by

A. Cytochrome p450
B. Cytochrome C
C. Cytochrome A
D. Both B and C

Answers

1. C **2.** A **3.** C **4.** A **5.** B **6.** B **7.** B **8.** B **9.** B **10.** C
11. A **12.** B **13.** D **14.** C **15.** A **16.** C **17.** B **18.** D **19.** B **20.** B
21. A **22.** D **23.** A **24.** B **25.** A

Chapter

4

Carbohydrates

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each
Case Studies: Marks: 4 or 5

INTRODUCTION

Carbohydrates are defined as the aldehydic or ketonic derivatives of higher polyhydroxy alcohol or anhydrides of such derivatives. Humans and all animals except carnivorous derive a major portion of their food calories from the various types of carbohydrates in their diets. Most of the energy for the metabolic activities of the cell in all organisms is derived from the oxidation of monosaccharide glucose.

In the form of glycogen (in the liver and muscles) carbohydrates act as an important food reserve. Carbohydrates are present in fruits in various forms such as monosaccharides, disaccharides, and polysaccharides. In the form of fibers in fruits, vegetables, and whole grains, carbohydrates are useful for regular bowel movements and in minimizing constipation-related disorders, and are useful to regulate blood sugar. Carbohydrates also serve as an important component of the red blood cell membrane. Disturbances in carbohydrate metabolism may lead to diabetes mellitus.

FUNCTIONS OF CARBOHYDRATES

Competency achievement: The student should be able to:

BI3.1: Discuss and differentiate monosaccharides, disaccharides, and polysaccharides giving examples of main carbohydrates as energy fuel, structural element and storage in the human body

BAQ: Write the definition and enumerate four important functions of carbohydrates.

Ans: Definition: Carbohydrates are defined as the aldehydic or ketonic derivatives of higher polyhydroxy alcohol or anhydrides of such derivatives.

Functions

A. Humans and all animals except carnivorous derive a major portion of their food calories from the various types of carbohydrates in their diets.
B. Most of the energy for the metabolic activities of the cell in all organisms is derived from the oxidation of carbohydrates (in the form of glucose).
C. In the storage organs of plants (in the form of starch) and in the liver and muscles of animals (in the form of glycogen), they are important food reserves.
D. Carbohydrates also serve as an important component of cell wall structure.

CLASSIFICATION AND PROPERTIES OF CARBOHYDRATES

BAQ: Give a classification of carbohydrates using a line diagram.

Ans:

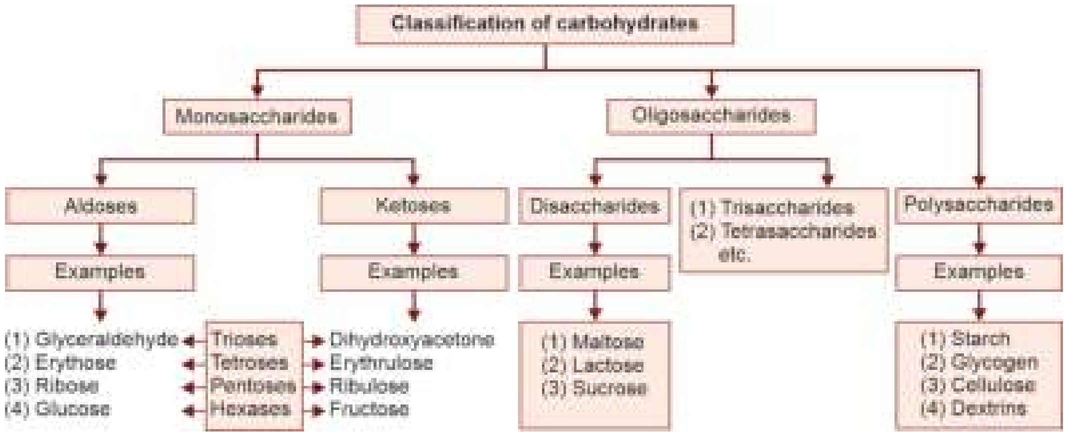

Classification of carbohydrates
Monosaccharides
Oligosaccharides
Aldoses
Ketoses
Disaccharides
(1) Trisaccharides
(2) Tetrasaccharides
etc.
Polysaccharides
Examples
Examples
Examples
Examples
(1) Glyceraldehyde
(2) Erythose
(3) Ribose
(4) Glucose
Trioses
Tetroses
Pentoses
Hexases
Dihydroxyacetone
Erythrulose
Ribulose
Fructose
(1) Maltose
(2) Lactose
(3) Sucrose
(1) Starch
(2) Glycogen
(3) Cellulose
(4) Dextrins

Fig. 4.1: Classification of carbohydrates

BAQ: What are monosaccharides? Give examples, using appropriate diagrams.

Ans: Monosaccharides consist of single polyhydroxy aldehyde or ketone units which cannot be broken down to simpler substances on hydrolysis. Monosaccharides containing an aldehyde group as the functional group are called aldoses, and those containing a ketone group as the functional group are called ketoses.

Carbohydrates are further classified according to the number of carbon atoms present. Monosaccharides containing three to seven carbon atoms are called trioses, tetroses, pentoses, and hexoses, respectively (Fig. 4.2).

Examples:

Aldose, hexose: Glucose and galactose
Ketose, hexose: Fructose
Aldose, pentose: Ribose
Ketose, pentose: Ribulose
Aldose, triose: Glyceraldehyde
Ketose, triose: Dihydroxyacetone

SAQ: What are oligosaccharides? Give suitable examples.

Ans: Oligosaccharides are defined as carbohydrates that contain between two and ten monosaccharide units per molecule joined by glucosidic linkages (Fig. 4.3)

On hydrolysis, they yield monosaccharides. Depending upon the number of constituent monosaccharide units, the oligosaccharides are called disaccharides, trisaccharides, etc.

BAQ: What are disaccharides? Give suitable examples.

Ans: Disaccharides consist of two monosaccharides joined by a glycosidic linkage. The most common and important disaccharides are maltose, lactose, and sucrose.

Maltose consists of two molecules of D-glucose joined by α-(1, 4)-glycosidic linkage. It is an important intermediate product of the digestion of starch and glycogen.

Lactose consists of galactose and glucose joined by α-(1, 4)-glycosidic linkage. It is a sugar present in milk to the extent of 5%.

Sucrose consists of glucose and fructose joined by α(1)–β(2)-glycosidic linkage. It is a plant disaccharide and is present in high concentrations in sugarcane and beet.

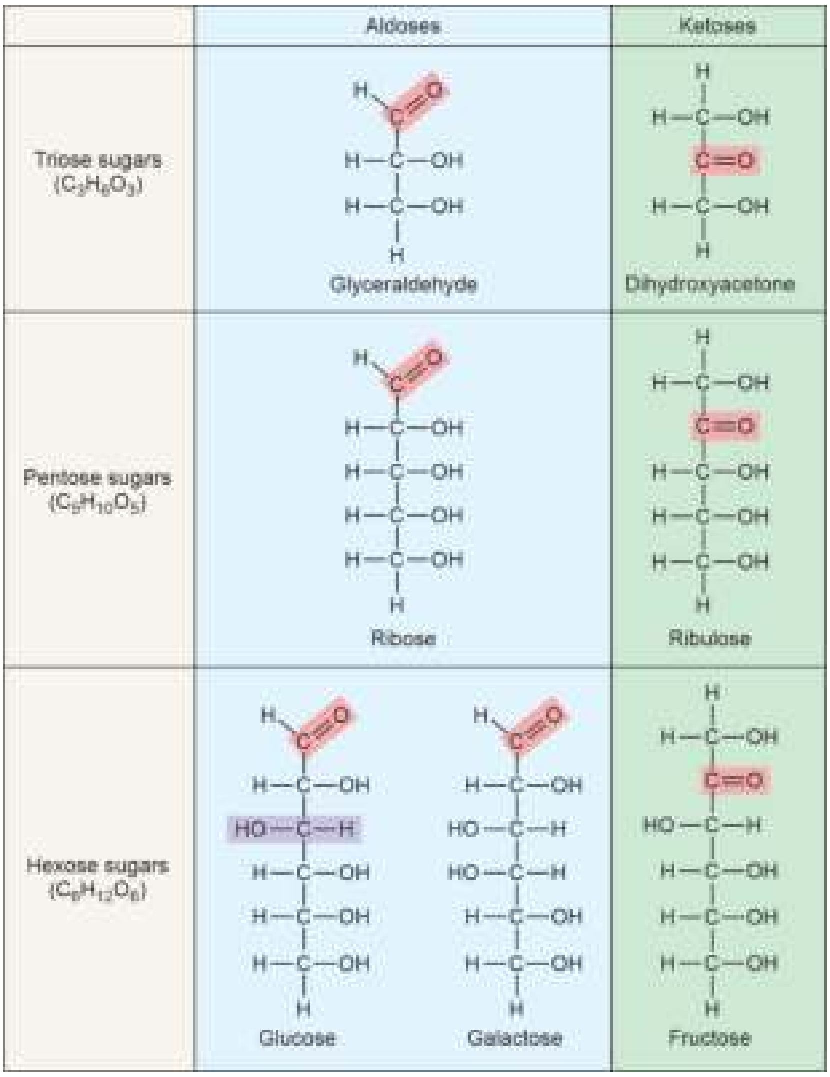

Aldoses
Ketoses
Triose sugars ($C_3H_6O_3$)
Glyceraldehyde
Dihydroxyacetone
Pentose sugars ($C_5H_{10}O_5$)
Ribose
Ribulose
Hexose sugars ($C_6H_{12}O_6$)
Glucose
Galactose
Fructose

Fig. 4.2: Aldoses and ketoses

Q: What are polysaccharides? Give suitable examples, using a diagram.

Ans: Polysaccharides are polymers of monosaccharide units that are joined in linear or branched chain fashion by glycosidic linkages. Polysaccharides contain a large number of sugar components per free carbonyl group (Fig. 4.3).

Polysaccharides can be divided into

A. Homopolysaccharides and

B. Heteropolysaccharides.

A. Homopolysaccharides: They contain only one type of monosaccharide as the repeating unit. On hydrolysis, they give only one type of sugar.

Examples: Starch, cellulose, glycogen, dextrin, etc.

B. Heteropolysaccharides: These are made up of mixed disaccharides repeating units and by hydrolysis, give a mixture of more than one product of monosaccharides and their derivatives.

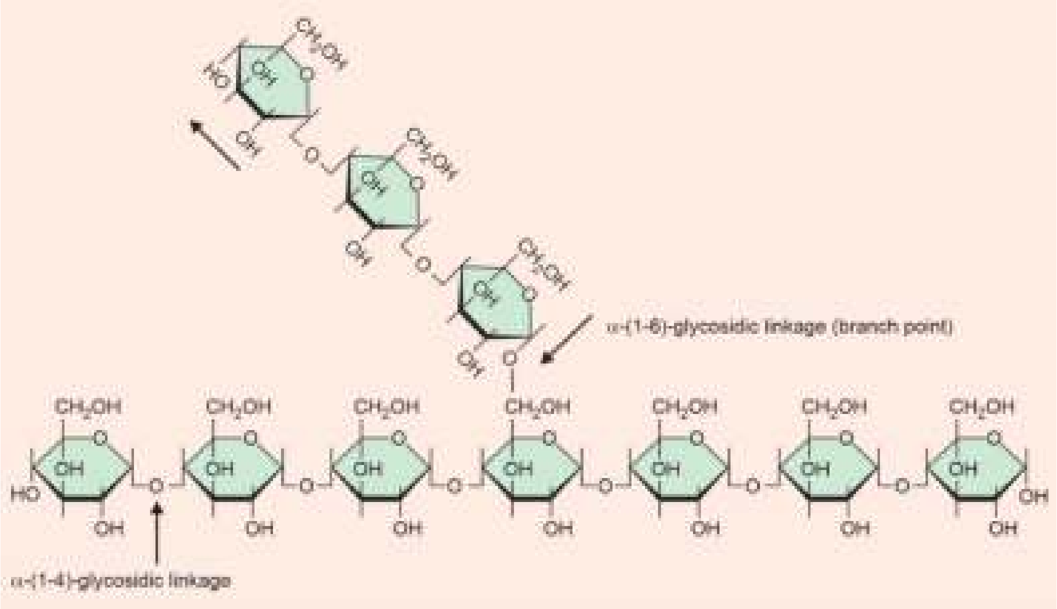

α-(1-6)-glycosidic linkage (branch point)
α-(1-4)-glycosidic linkage
CH₂OH
OH
HO
O

Fig. 4.3: Basic polysaccharide structure

Examples: Mucopolysaccharides, which include hyaluronic acid, heparin, chondroitin sulfate, dermatan sulfate, and keratan sulfate.

SAQ: What are mucopolysaccharides?

Ans: Mucopolysaccharides, also known as glycosaminoglycans, are complex carbohydrates containing amino sugars and uronic acids. They are attached to protein molecules to form proteoglycans, which provide the packing substance of connective tissue.

SAQ: Write two important functions of hyaluronic acid in the body.

Ans: A. Hyaluronic acid present in joints is useful for smooth movements of bone joints and prevents injury from bones grinding against each other.

B. It is capable of holding water molecules and keeps the surroundings hydrated.

SAQ: Why hyaluronic acid is used in pharmaceutical products?

Ans: Hyaluronic acid makes the skin flexible and reduces skin wrinkles and lines. Hyaluronic acid also plays an important role in the fast healing of wounds and can reduce scarring.

SAQ: How heparin is synthesized in the body? Write two important functions of heparin in the body.

Ans: Heparin is synthesized by specific white blood cells known as mast cells. Glucuronic acid and glucosamine molecules are used in the synthesis of heparin.

Functions

1. Heparin prevents the formation of blood clots in blood circulation.
2. It plays an important role in the reduction of formed blood clots already formed in blood circulation.

SAQ: Write uses of heparin in a clinical laboratory.

Ans: Heparin is used as a very important anticoagulant for blood tests. It prevents blood clotting. Anticoagulated blood is then used for many emergency laboratory tests. Heparin is also used for the separation of plasma for several clinical laboratory tests.

SAQ: Write a short note on chondroitin sulfate.

Ans: Chondroitin sulfate is a major constituent of various tissues such as skin, bone, cartilage, cornea, tendons, valves, etc. It helps to maintain the shape of the organ and helps to provide resistance to compression. Chondroitin is used as a component of dietary supplements to treat osteoarthritis.

SAQ: What are dermatan sulfate and its functions?

Ans: Dermatan sulfate is an important component of skin, lungs, tendons, blood vessels, heart valves, tendons, etc. It helps to maintain the shape of the component in which it is present.

SAQ: What are keratan sulfate and its functions?

Ans: Keratan sulfate is present in the cornea, bones, cartilage, and central nervous system. It maintains the transparent nature of the cornea and has a neuroprotective role in the central nervous system.

SAQ: Name one disorder related to mucopolysaccharides.

Ans: Mucopolysaccharidosis is a genetic lysosomal storage disorder, in which, due to congenital deficiency of specific enzymes, mucopolysaccharides are not degraded and a high amount of mucopolysaccharides are excreted in the urine. Children with this disorder suffer from intellectual disability and physical health problems.

SAQ: Name four carbohydrates that form components of blood group antigen chains and are responsible for blood group differentiation.

Ans: The carbohydrates present in the blood group antigen chains are N-acetylgalactosamine, N-acetylglucosamine, D-galactose, and fucose.

SAQ: What is glycophorin and its functions?

Ans: Glycophorin is a major integral membrane glycoprotein of human erythrocytes. Glycophorins are rich in sialic acid, which gives the red cells a very hydrophilic-charged coat. This enables them to circulate without adhering to other cells or vessel walls.

SAQ: What is the function of the carbohydrate sialic acid as a part of the red blood cell membrane?

Ans: Sialic acids are a class of alpha-keto acid sugars present in various tissues and are integral membranes of red blood cells.

BAQ: Give an account of two properties of carbohydrates that are useful to identify carbohydrates in body fluids.

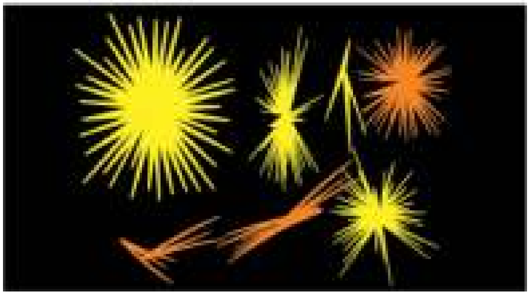
Ans: Following are the important properties of some of the carbohydrates on which clinical chemistry methods are based:

1. **Reducing property:** Monosaccharides and disaccharides having free aldehydic or ketonic groups in their structure, reduce metallic cations, such as cupric ions in an alkaline solution such as Benedict's reagent or Fehling's reagent at high temperatures to form a cuprous oxide of various colors. The original blue color of these reagents changes to green, yellow, orange, or red color, depending on the concentration of reducing sugar present in the specimen.

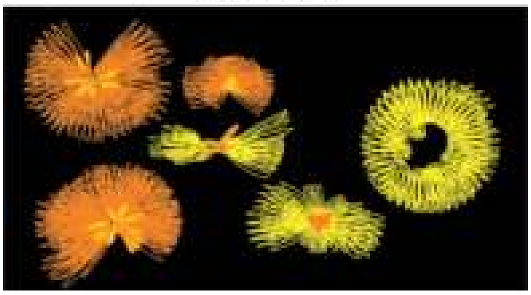
   All the monosaccharides and the two disaccharides, maltose, and lactose, reduce cupric ions present in Benedict's reagent and also in Fehling's reagent. In the test change of color of the reagent indicates the presence of a reducing substance in the specimen like urine.
2. **Formation of osazone crystals:** The reducing sugars (all monosaccharides, lactose, and maltose) form characteristic osazone crystals. These are obtained by adding a mixture of phenylhydrazine hydrochloride and sodium acetate to the sugar solution and then by heating the mixture in a boiling water bath. These compounds have characteristic crystal structures, melting points, and precipitation times, and are valuable in the identification of sugars.

Glucose, fructose, and mannose give the same types of osazones (needle-shaped), maltose forms sunflower-type crystals and lactose forms cotton ball types of osazones. Hence, lactose can be differentiated from other reducing substances, if present in urine.

Osazones formed by glucose, fructose, and mannose



Maltosazones



Lactosazones

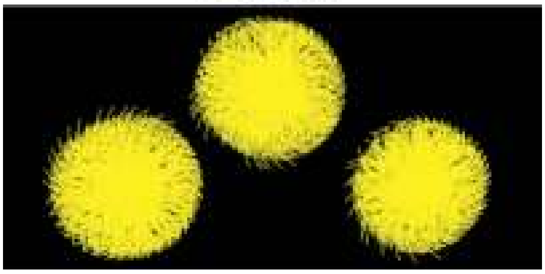


Fig. 4.4: Various osazone crystals

DIGESTION AND ABSORPTION OF CARBOHYDRATES

Competency achievement: The student should be able to:

BI3.2 and 3.3: Describe the process involved in the digestion and assimilation of carbohydrates from food

Q: Name carbohydrates present in the following food items: Apples, grapes, honey, sugarcane, milk, rice, wheat bread, meat and sea food.

Ans:

Food item	Carbohydrate content
Apples	Mainly fructose
Grapes	Glucose and fructose
Honey	Glucose, fructose and sucrose
Sugarcane	Sucrose
Milk	Lactose
Rice	Starch
Wheat bread	Starch
Meat, sea food	Glycogen

LAQ: Describe digestion and absorption of carbohydrates.

Ans: About 50 to 60 percent of the daily caloric intake is supplied by carbohydrates. This is equivalent to 250 to 500 gm in the form of commonly digestible carbohydrates such as polysaccharides, disaccharides, and assimilable monosaccharides.

The main sources of polysaccharides in the form of starch are cereals, grains, vegetables, and tubers. Meat and seafood contain small quantities of glycogen, i.e. another form of polysaccharide. Disaccharides such as lactose and sucrose are present in milk and sugarcane respectively. Monosaccharides such as glucose and fructose are present in various types of fruits.

In the oral cavity, salivary amylase is capable of bringing about the hydrolysis of starch in the food to maltose and dextrins. This part of digestion in the oral cavity is of a little significance because of the short time the salivary amylase can act on the food.

Further digestion of carbohydrates takes place in the duodenum by pancreatic amylase. Starch and dextrins are further hydrolyzed to maltose, maltotriose, and a mixture of branched and non–branched oligosaccharides and some glucose at an alkaline pH of 7.5–8.0.

The intestinal juice secreted by the glands of Brunner and Lieberkuhn contains various enzymes such as maltase which removes single glucose residues from α-(1–4) linked oligosaccharides and disaccharides. Isomaltase hydrolyses, 1–6 bonds in α-limit dextrins. β-galactosidase removes galactose from lactose and sucrase hydrolyses sucrose into glucose and fructose. β-glucosidase catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in D-glucosides and oligosaccharides with the release of glucose. The end products of carbohydrate digestion are glucose, fructose, mannose, galactose, and pentose sugars.

The end products of the digestion of carbohydrates are mainly the monosaccharides, glucose, galactose, and fructose (and pentoses, if present in food).

The comparative rates of absorption of monosaccharides taking glucose as 100 may be indicated as follows:

Galactose (110), glucose (100), fructose (43), mannose (39), xylose (15), and arabinose (9). Galactose and glucose are absorbed at a faster rate compared to fructose and pentose. This is because glucose and galactose are actively transported while fructose, mannose, and pentose are absorbed by simple diffusion.

BAQ: Describe the active transport of glucose and galactose.

Ans:

1. Sodium ions and a mobile carrier are required to transport glucose and galactose through the plasma membrane of the intestinal cells.

   The mobile carrier binds both glucose and Na^+ at separate sites and then these are transported against a concentration gradient from the intestinal lumen to cytosol.
2. The free energy required for this active transport is obtained from the hydrolysis of ATP molecules, which expel Na^+ from the cell.
3. The monosaccharides are absorbed into the mucosal cells of the small intestine and pass into circulation via the portal vein.

   The microvilli (brush border) lining the mucosa cells greatly help the absorption by increasing the surface area.

SAQ: Define the glycemic index (GI).

Ans: The glycemic index (GI) is a measure of the effects of carbohydrates on blood glucose levels. GI determines how much each gram of available carbohydrate in a portion of food increases the blood glucose of a person following foods relative to consumption of glucose, which has a GI of 100.

SAQ: Which carbohydrates have a high glycemic index (GI)? Give examples.

Ans: Foods with carbohydrates that breakdown quickly during digestion and release glucose rapidly into the blood have a high GI. Examples: Glucose, maltose, white rice, cornflakes, white bread, etc. These all types have GI >70.

SAQ: Which carbohydrates have a low glycemic index (GI)? Give examples.

Ans: Foods that breakdown slowly releasing glucose into the blood have low GI. Examples: Fructose, most fruits, vegetables, legumes, pulses, beet, etc. These food have GI <55. The following types of food have intermediate GI (56–69): Sucrose, sweet potato, whole wheat products, etc.

Clinical Conditions Associated with the Absorption of Carbohydrates

BAQ: What is lactose intolerance? What are the symptoms related to lactose intolerance?

Ans: Some patients have a congenital or acquired deficiency of lactase. Hence, lactose present in food (milk and milk products) is not digested. The intestine normally is impermeable to disaccharides. The presence of undigested disaccharide causes increased fluid secretion into the gut and increased intestinal motility. Lactose is fermented by enteric bacteria, and hydrogen, carbon dioxide, and organic acids (e.g. lactic acid) are produced. This causes abdominal discomfort, such as bloating, distension, and cramping.

In the large bowel, the presence of carbon dioxide and organic acids decreases pH and keeps the osmolality high, and decreases water reabsorption. The result is acidic and liquid stool diarrhea.

SAQ: What is the difference between congenital and acquired deficiency of lactase?
Ans: Congenital lactase deficiency is inborn. Acquired lactase deficiency is caused due to loss of brush-border lactase in the small intestine due to diarrhea, which may be infective, drug-induced, or caused due to antibiotic intolerance. Acquired lactase intolerance may be of short duration.

SAQ: How lactose intolerance is detected? What laboratory test is performed? Write the principle of the test. What is the treatment to cure lactose intolerance?
Ans: Persistent crying of a baby following milk intake may be due to abdominal discomfort and cramps related to the fermentation of lactose. Lactose intolerance can be detected by an osazone test in a clinical laboratory.

Principle of osazone test: **Refer to p40.**

Treatment: Patients are advised to avoid milk and milk products.

CARBOHYDRATE METABOLISM

Competency achievement: The student should be able to:
BI3.4: Define and differentiate the pathways of carbohydrate metabolism: Glycolysis, gluconeogenesis, glycogen metabolism, and HMP shunt

SAQ: What is glucose metabolism?

Ans: Glucose metabolism means: Once in the blood stream, absorbed monosaccharides form a common glucose pool. Glucose molecules then get distributed to the cells of the body, where they undergo many remarkable biochemical changes. The total of these changes has been named metabolism. It includes (1) Energy requiring synthesis of new complex organic compounds, such as glycogen (anabolism) and (2) Energy releasing degradation of absorbed nutrients to simple end products such as carbon dioxide and water (catabolism**)**.

SAQ: What is the significance of carbohydrate metabolism?

Ans: The major function of carbohydrates in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes. After digestion, absorbed monosaccharides form a common glucose pool. Glucose molecules then get distributed to the cells of the body. Glucose molecules supply more than 50% of the energy requirement of the body. Much of the dietary carbohydrate is converted to fat and consequently metabolized as fat.

BAQ: What are the various metabolic pathways of carbohydrates? Write the significance of each metabolic pathway.
Ans: The metabolism of carbohydrates can be subdivided as follows:

1. Glycolysis,
2. Glycogenesis,
3. Glycogenolysis
4. The citric acid cycle: (Krebs' cycle),
5. The hexose monophosphate shunt: (HMS, direct oxidative pathway), and
6. Gluconeogenesis. The significance of each of these pathways is as follows:
    1. *Glycolysis:* The oxidation of glucose or glycogen to pyruvate and lactate by the Embden-Meyerhof pathway.
    2. *Glycogenesis:* The synthesis of glycogen from glucose.
    3. *Glycogenolysis:* The conversion of glycogen to glucose.
    4. *The citric acid cycle* (Krebs' cycle): The final common pathway of oxidation of glucose, fatty acids, and amino acids through which acetyl-CoA is completely oxidized to CO_2 and water.
    5. *The hexose monophosphate shunt:* (HMS, direct oxidative pathway): An alternative pathway to the Embden-Meyerhof pathway and citric acid cycle for the oxidation of glucose to CO_2, and water.

6. *Gluconeogenesis:* The formation of glucose from noncarbohydrates such as glycerol, pyruvic acid, lactic acid, etc.

SAQ: What is glucose catabolism?

Ans: Glucose catabolism means a breakdown of glucose molecules to get energy in the form of ATP molecules. Glucose catabolism is one of the primary metabolic events that occur during cell metabolism.

SAQ: What is cellular respiration?

Ans: The portion of cell metabolism that breaks down glucose is generally called cellular respiration. Cellular respiration has three major events: Glycolysis, the Krebs cycle, and the electron transport system (ETS).

SAQ: What are the locations of the major metabolic pathways of carbohydrates in a cell?

Ans:

Metabolic pathway	**Location**
Glycolysis	Cytoplasm
Krebs cycle	Mitochondria (matrix)
Electron transport system	Mitochondria (cristae)

BAQ: What is glycolysis? Enumerate important features of glycolysis.

Ans: Glycolysis is defined as the conversion of glucose or glycogen to pyruvate or lactate through a series of enzyme-catalyzed reactions with the production of ATP molecules. Glycolysis takes place in all the cells of the body. It can take place anaerobically in the absence of oxygen or aerobically in the presence of oxygen. In anaerobic oxidation of glucose pyruvic acid is the end product with ATP molecules and in aerobic oxidation glucose is converted to pyruvic acid, which is oxidized to carbon dioxide and water molecules with the production of ATP molecules.

The important features of glycolysis are as follows:

1. Glycolysis is a major pathway in red blood cells, the lens, and the cornea of the eye for the supply of ATP molecules.
2. Glycolysis is essential for the functions of the brain, which require ATP molecules.
3. Intermediate products formed in glycolysis are useful for fat and amino acid synthesis.
4. Glycolysis reversal through specific steps of metabolism leads to the formation of glucose (gluconeogenesis).

BAQ: Describe the energetics of glycolysis.

Ans: Tissues are mainly dependent on glucose for their metabolic activities. Brain and red blood cells are completely dependent on glucose for their functions. Glycolysis occurs in the cytosol of all cells. It can function either anaerobically or aerobically, depending on the availability of oxygen and the electron transport chain.

In phase 1 of glycolysis, only two ATP molecules are obtained in the anaerobic oxidation of glucose and the end product is pyruvic acid which is converted to lactic acid by the catalytic action of lactic dehydrogenase.

In the next two phases, in aerobic oxidation, glucose is completely oxidized to carbon dioxide and water which requires both oxygen and the mitochondrial enzyme system comprised of pyruvate dehydrogenase complex, citric acid cycle, and the respiratory chain. In the aerobic oxidation of glucose, 36 ATP molecules are obtained.

Embden-Meyerhof Pathways

BAQ: What is the investment phase of the Embden-Meyerhof pathway?

Ans: During the first step of glycolysis, phosphorylation of glucose takes place by a specific enzyme called hexokinase to form glucose 6-phosphate (G6-P). This reaction requires one ATP molecule.

In the next step, G6-P is converted into fructose 6-phosphate (F6-P) by glucose phosphate isomerase. The reaction is catalyzed by an enzyme, phosphohexose isomerase.

The enzyme phosphofructokinase (PFK) then acts on F6-P in the presence of magnesium ions and one ATP to form fructose 1:6 biphosphate and ADP.

Overall, 2 ATP molecules are consumed (or used) in this phase of glycolysis, hence this phase is called the investment phase.

SAQ: What are the Embden-Meyerhof pathways? What is the significance of Embden-Meyerhof pathways?

Ans: Embden-Meyerhof (EM) pathways are the series of enzymatic reactions in the anaerobic conversion of glucose to lactic acid, resulting in energy in the form of two adenosine triphosphate (ATP).

EM pathways are important since in the absence of oxygen, tissues can derive energy from oxidation of glucose to ATP molecules. Similarly, red blood cells do not contain mitochondria and are completely dependent on EM pathways to get ATP molecules for their activities. The brain is also able to get ATP molecules in hypoxia.

LAQ: Describe the steps of the Embden-Meyerhof pathways of glucose metabolism (Fig. 4.5).

Ans: Anaerobic glycolysis takes place by Embden-Meyerhof pathways of glucose metabolism. Anaerobic glycolysis is particularly very important because it provides ATP molecules to skeletal muscle in hypoxia and then tissues can survive anoxic episodes. Similarly, since red blood cells lack mitochondria, these are dependent on Embden-Meyerhof pathways for energy in the form of ATP molecules. The biochemical reactions of anaerobic glycolysis are given in the line diagram (Fig. 4.5):

Phase-1 (Investment phase)

A. During the first step of glycolysis phosphorylation of glucose takes place by a specific enzyme called hexokinases to form glucose 6-phosphate (G6-P). This reaction requires ATP and it is an irreversible reaction.

In the next step, G6-P is rearranged into fructose 6-phosphate (F6-P) by glucose phosphate isomerase. This is a reversible reaction

B. The enzyme phosphofructokinase (PFK) then acts on F6-P in the presence of magnesium ions and ATP to form fructose 1:6 biphosphate and ADP. If adequate ATPs are available, PFK is inhibited allosterically by ATP. However, a large amount of AMP activates PFK allosterically so that the formation of ATP molecules will take place.

This is an irreversible reaction and a rate-limiting and committed step. That means, from this step, the completion of EM pathways will take place.

C. The enzyme aldolase acts on fructose 1:6 biphosphate. At this stage, the hexose ring split splits into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde.

It is important to note here that, in phase-1(also known as the preparatory phase), two ATP are consumed hence it is known as the "investment phase".

Phase-2 (Pay-off phase)

- Two triose sugars, dihydroxyacetone phosphate (a ketone), and glyceraldehyde 3-phosphate (an aldehyde), are in equilibrium by the action of the enzyme phosphorylase isomerase. However, glyceraldehydes-3-phosphate is continuously removed by the next step of Glycolysis and hence dihydroxyacetone is continuously converted to glyceraldehydes-3-phosphate.
- The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1,3-bisphosphoglycerate. The enzyme glyceraldehydes 3-phosphate dehydrogenase catalyzes this reaction. The hydrogen is used to reduce two molecules of NAD^+, to give NADH + H^+ for each triose.
- The enzyme phosphoglycerate kinase in the presence of magnesium ions and ADP acts on 1, 3 biphosphoglycerate to form 3-phosphate glycerate and ATP.

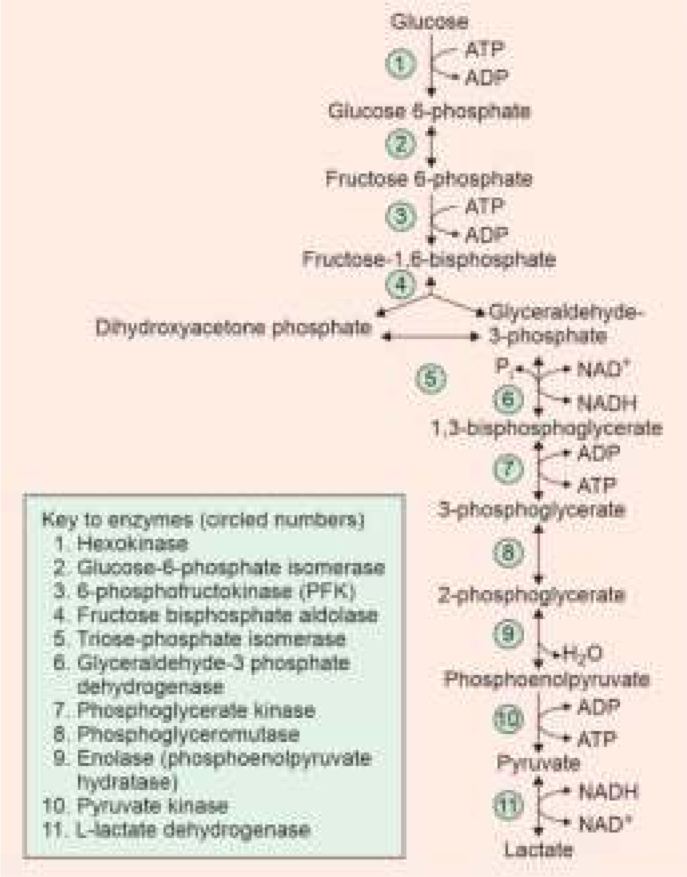

Glucose
① ATP ADP
Glucose 6-phosphate
②
Fructose 6-phosphate
③ ATP ADP
Fructose-1,6-bisphosphate
④
Dihydroxyacetone phosphate
Glyceraldehyde-3-phosphate
⑤
⑥ Pi NAD+ NADH
1,3-bisphosphoglycerate
⑦ ADP ATP
3-phosphoglycerate
⑧
2-phosphoglycerate
⑨ H2O
Phosphoenolpyruvate
⑩ ADP ATP
Pyruvate
⑪ NADH NAD+
Lactate
Key to enzymes (circled numbers)
1. Hexokinase
2. Glucose-6-phosphate isomerase
3. 6-phosphofructokinase (PFK)
4. Fructose bisphosphate aldolase
5. Triose-phosphate isomerase
6. Glyceraldehyde-3 phosphate dehydrogenase
7. Phosphoglycerate kinase
8. Phosphoglyceromutase
9. Enolase (phosphoenolpyruvate hydratase)
10. Pyruvate kinase
11. L-lactate dehydrogenase

Fig. 4.5: Embden-Meyerhof pathways

- Phosphoglycerate mutase now acts on 3-phosphoglycerate to form 2-phosphoglycerate.
- Enolase in the presence of magnesium ions acts on 2-phosphoglycerate to form phosphoenolpyruvate.
- Pyruvate kinase in the presence of magnesium ions and ADP acts on phosphoenol pyruvate to form pyruvate and ATP.
- In the muscles, lactate dehydrogenase (LDH) acts on pyruvate in the presence of coenzyme NADH to form lactate and NAD.
- The overall equation for glycolysis from glucose to lactic acid is as follows:
- Glucose + 2ADP + 2Pi → 2 lactate + 2ATP + $2H_2O$

It is important to note here that:

The phase-2 of glycolysis is known as the pay-off phase since there is a net gain of the energy-rich molecules 2ATP molecules and two NADH molecules.

Since glucose yields two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose.

If glycolysis starts from glycogen, the net gain will be three ATP molecules, since only one ATP is consumed.

In erythrocytes (due to lack of mitochondria), glycolysis is terminated in the formation of lactate. Other tissues that derive energy from glycolysis that terminates into the formation of lactate include the brain, retina, skin, gastrointestinal tract, and renal medulla. The heart, liver, and kidneys take up lactate and oxidize it in hypoxic conditions.

Competency achievement: The student should be able to:

BI3.7: Describe the common poisons that inhibit crucial enzymes of carbohydrate metabolism (e.g. fluoride, arsenate)

BAQ: Describe the common poisons that inhibit crucial enzymes of Embden-Meyerhof pathways.

Ans:

1. The toxicity of arsenic is the result of the competition of arsenic with inorganic phosphorus in reaction number 6 (Fig. 4.5).
2. Fluoride inhibits the enzyme enolase (step 9) (Fig. 4.5) and glycolysis subsequently. Hence in the blood glucose test, fluoride is used as one of the components of anticoagulants to prevent glycolysis.

SAQ: Give reason: Why congenital deficiency of pyruvate kinase leads to hemolytic anemia?

Ans: Absence of pyruvate kinase (step 10) (Fig. 4.5) in red blood cells leads to deprivation of ATP molecules for red blood cell growth and functions. A decrease in the life span of red blood cells leads to hemolytic anemia.

SAQ: Give reason: Why in fast-growing cancer cells a large amount of lactic acid is produced?

Ans: In fast-growing cancer cells a large amount of lactic acid is produced due to the high rate of growth of cancer cells and that of a related high rate of glycolysis.

SAQ: Give reason: Why conversion of NADH to NAD is important?

Ans: NAD is present in small amounts at the cellular level. Since NAD is required for the continuation of glycolysis, NADH must be converted back to NAD. Hence in the muscles, once pyruvate is formed, LDH acts on it in the presence of NADH to form lactate and NAD. Lactate is converted to glucose in the liver later on by gluconeogenesis.

SAQ: Give reason: Why deficiency of thiamine leads to lactic acidosis?

Ans: Pyruvate in the cytosol is transported to mitochondria and it is oxidatively decarboxylated in the Krebs cycle to acetyl-CoA using thiamine as a coenzyme. A deficiency of thiamine leads to the accumulation of pyruvate, leading to pyruvic and lactic acidosis.

SAQ: Why high intake of fructose leads to obesity?

Ans: Fructose enters glycolysis by phosphorylation to fructose 1-phosphate and bypasses the main regulatory steps. In this process, more pyruvic acid and acetyl-CoA are formed, which leads to increased lipogenesis. Hence intake of high sucrose and fructose (and fructose corn syrup in cold drinks) containing food and drinks leads to obesity.

SAQ: What is the importance of the formation of 2,3-biphosphoglycerate in the tissues?

Ans: In erythrocytes, the reaction catalyzed by phosphoglycerate kinase may be bypassed to some extent, which leads to the formation of 2,3-biphosphoglycerate. In the tissues, 2,3-biphosphoglycerate binds to hemoglobin, decreasing its affinity to oxygen, and then oxygen is available to the cells.

SAQ: What is substrate-level phosphorylation?

Ans: Substrate-level phosphorylation means the formation of ATP (or GTP) by the direct transfer and donation of a phosphoryl (PO_3) group to ADP (or GDP) from ATP (or GTP). In the pay-off phase, two ATP molecules are formed by substrate-level phosphorylation.

SAQ: What is the role of vitamin B_3 (niacin) in EM pathways?

Ans: Niacin is present in the oxidized form as nicotinamide adenine dinucleotide (NAD) and in the reduced form as nicotinamide adenine dinucleotide hydrogen (NADH) at the cellular level. NAD acts as a coenzyme for the activity of the enzyme lactate dehydrogenase (LDH). In the muscles, once pyruvate is formed, LDH acts on it in the presence of NADH to form lactate and NAD. Lactate is then converted to glucose in the liver to maintain normal glucose levels in fasting conditions.

SAQ: What is the Pasteur effect?

Ans: The Pasteur effect means the inhibition of anaerobic glycolysis of glucose by the presence of oxygen. The presence of oxygen favors aerobic glucose oxidation for more gain of ATP molecules (about 36). Only two ATP molecules are produced in anaerobic glycolysis.

SAQ: What is the Crabtree effect?

Ans: Inhibition of aerobic oxidation of glucose by the addition of glucose molecules to tissues is known as the Crabtree effect. The capability to ferment sugars into ethanol is one important metabolic trait of yeasts. For the manufacture of ethanol, the Crabtree effect is useful since yeasts use fermentation of glucose to ethanol reaction in the presence of oxygen.

BAQ: What is the Rapoport-Leubering cycle and its significance?

Ans: The Rapoport-Leubering cycle is responsible for the synthesis of 2,3-biphosphoglycerate in matured red blood cells. 1,3-biphosphoglycerate formed in glycolysis is converted to 2,3-biphosphoglycerate by the action of the enzyme 2,3-biphosphoglycerate mutase. About 20% of the glucose that is converted to lactate is used in the synthesis of 2,3-biphosphoglycerate, which combines with hemoglobin in red blood cells and reduces the ability of hemoglobin to combine with oxygen. Thus, when oxygenated blood gets circulated in tissues, oxygen is released to the cells, due to hemoglobin-2,3-biphosphoglycerate combination.

SAQ: What is the effect of anaerobic glycolysis in the oral cavity by the anaerobic bacteria in dental caries?

Ans: Anaerobic bacteria like *streptococcus mutans* use glucose for ATP molecules for their growth. The end product of anaerobic glycolysis is lactic acid, which acts on teeth, leading to dental caries.

KREBS CYCLE (TCA CYCLE OR CITRIC ACID CYCLE)

SAQ: What is the TCA cycle (Krebs cycle) and its significance?

Ans: The Krebs cycle, also known as the citric acid cycle or tricarboxylic acid (TCA) cycle, is a series of chemical reactions in the cell that breaks down food molecules into carbon dioxide, water, and energy. The Krebs cycle is named after Hans Krebs and is a metabolic event that follows Glycolysis. This process occurs in the fluid matrix of the mitochondrion. In this process, pyruvic acid obtained from glycolysis is used and the process is aerobic. The citric acid cycle is the common pathway for the oxidation of glucose, fatty acids, and most amino acids since these are metabolized to acetyl-CoA or intermediates of the citric acid cycle. It also plays an important role in the lipogenesis, gluconeogenesis, and interconversion of nonessential amino acids.

Competency achievement: The student should be able to:

BI3.6: Describe and discuss the concept of the TCA cycle as an amphibolic pathway and its regulation

LAQ: Describe the TCA cycle (Krebs cycle) using a suitable diagram.

Ans: The Krebs cycle follows glycolysis (Fig. 4.6). This process occurs in the fluid matrix of the mitochondrion. In this process, pyruvic acid obtained from glycolysis is used and the process is aerobic.

In the first step of the Krebs cycle, pyruvic acid is converted to acetyl-CoA.

The conversion of pyruvic acid to acetyl-CoA takes place in a three-step process as follows:

1. Each of the two pyruvic acid molecules is decarboxylated and at this point, two carbon dioxide molecules are produced and these diffuse to the blood. This event yields two acetyl groups.
2. In the next step, hydrogen is removed from each acetyl group and added to NAD. The removal of hydrogen is called dehydrogenation which is an oxidation process. The addition of hydrogen to NAD is a reduction process.
3. Finally, CoA is added to each acetyl group.

   Acetyl-CoA which results from the conversion of pyruvic acid then reacts with oxaloacetate using an enzyme called citrate synthase. This results in the formation of citric acid, the first major product of the Krebs cycle. Because of this reaction, the Krebs cycle is sometimes called the citric acid cycle. The citric acid is then systematically decarboxylated and dehydrogenated to use up the acetyl groups that were attached to the oxaloacetate. This allows oxaloacetate and CoA to be used in the next cycle.

   - The conversion of citric acid back to oxaloacetate involves three dehydrogenations that form three reduced NAD ($NADH_2$) molecules, and one dehydrogenation that forms one reduced FAD ($FADH_2$). Similarly, two decarboxylation reactions from two carbon dioxide molecules and one substrate phosphorylation form an ATP molecule. When two acetyl-COA molecules are utilized, two cycles occur and the above output is doubled.

Various reactions involved in the citric acid cycle are as follows:

- Oxaloacetate + Acetyl-CoA + $H_2O \rightarrow$ Citrate + CoA-SH. This reaction (exothermic) is catalyzed by citrate synthase with the formation of citrate and CoA-SH.
- Citrate $\rightarrow$ *cis*-Aconitate + H_2O. The enzyme aconitase isomerizes citrate to form cis-Aconitate.
- *cis*-Aconitate + $H_2O \rightarrow$ Isocitrate. The enzyme aconitase also acts on cis-Aconitate to form isocitrate.
- Isocitrate + $NAD^+ \rightarrow$ Oxalosuccinate + NADH + H^+. Isocitrate undergoes dehydrogenation by the action of isocitrate dehydrogenase
- Oxalosuccinate + α-Ketoglutarate + CO_2. Oxalosuccinate undergoes decarboxylation in the presence of magnesium ions and this reaction is catalyzed by isocitrate dehydrogenase.
- α-Ketoglutarate + NAD^+ + CoA-SH $\rightarrow$ Succinyl-CoA + NADH + H^+ + CO_2. α-Ketoglutarate undergoes oxidative decarboxylation and the reaction is catalyzed by α-ketoglutarate dehydrogenase, which requires the cofactors comprised of thiamine diphosphate, lipoate, NAD, FAD, and CoA.
- Succinyl-CoA + GDP + Pi $\rightarrow$ Succinate + CoA-SH + GTP. Succinyl-CoA synthetase acts on succinyl-CoA in the presence of GDP and Pi to form succinate.
- Succinate + ubiquinone (Q) $\rightarrow$ Fumarate + ubiquinol (QH_2). Succinate dehydrogenase is bound to the inner membrane of mitochondria. It acts on succinate in the presence of ubiquinones to form fumarate.
- Fumarate + $H_2O \rightarrow$ L-Malate. The enzyme fumarase acts on fumarate to form L-malate.
- L-Malate + $NAD^+ \rightarrow$ Oxaloacetate + NADH + H^+. The enzyme malate dehydrogenase acts on L-malate in the presence of the coenzyme NAD to form oxaloacetate, which again enters the citric acid cycle.
- The overall reaction for the citric acid cycle is as follows:
- Acetyl-CoA + $3NAD^+$ + FAD + GDP + Pi + $2H_2O \rightarrow FADH_2 + 3NADH + 3H^+$ + CoA + GTP + $2CO_2$

In this reaction, Q is ubiquinone and Pi is inorganic phosphate.

Table 4.1: Output of the Krebs cycle
• Reduced coenzymes: 3NADH + 1 $FADH_2$
• Reoxidation of 3NADH + 1 $FADH_2$ = 9 ATP
• Substrate level phosphorylation = 1 ATP (or GTP)
• Total ATPs = 10

It is important to note the following aspects of the TCA cycle:

1. Following four B-complex vitamins are essential in the citric acid cycle: Riboflavin (in the form of FAD), niacin (in the form of NAD), thiamine (as thiamine diphosphate), and pantothenic acid (as a part of coenzyme A).
2. TCA is also a major pathway for the interconversion of metabolites arising from transamination and deamination as well as for gluconeogenesis and fatty acid synthesis.
3. TCA is known as amphibolic since it functions in both oxidative and synthetic processes (both catabolic and anabolic reactions).

SAQ: What is the action of fluoroacetate on the TCA cycle?

Ans: Fluoroacetate is a poisonous substance. It inhibits the enzyme aconitase leading to the accumulation of citric acid. The enzyme aconitase carries out the following reaction in TCA:

cis-Aconitate + H_2O → Isocitrate. The enzyme aconitase also acts on *cis*-Aconitate to form isocitrate.

SAQ: What are the clinical disorders related to the TCA cycle?

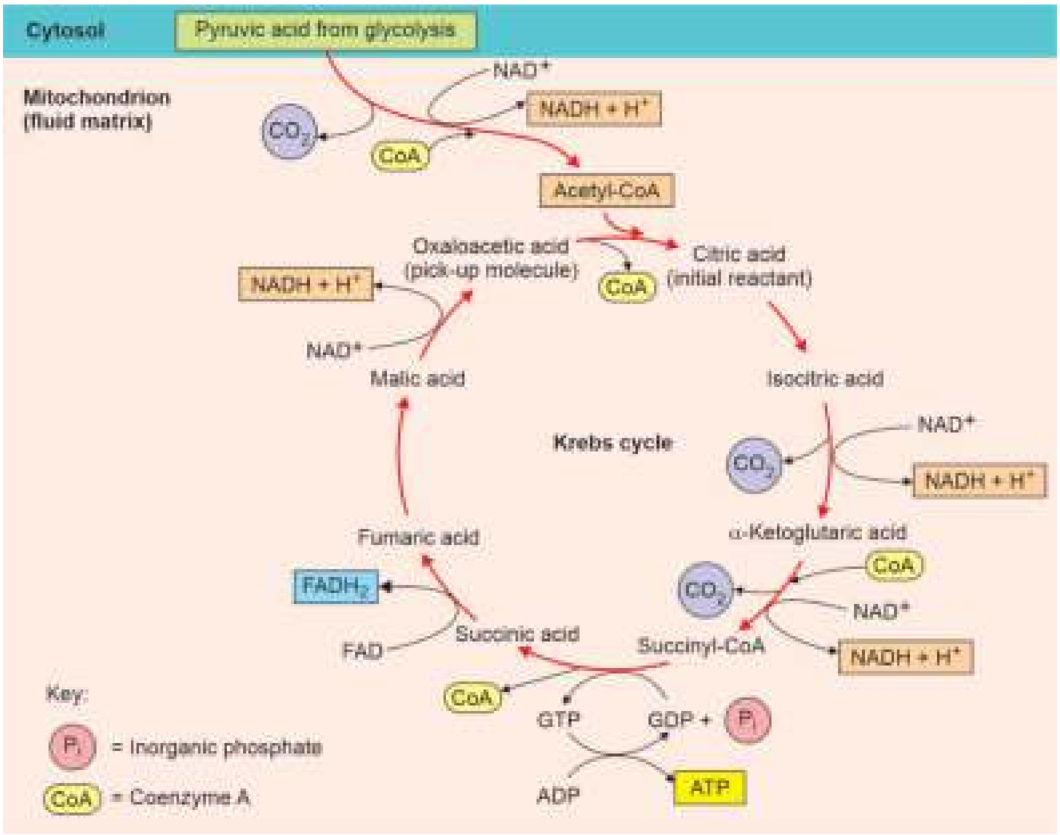

Cytosol
Pyruvic acid from glycolysis
Mitochondrion (fluid matrix)
NAD^+
$NADH + H^+$
CO_2
CoA
Acetyl-CoA
Oxaloacetic acid (pick-up molecule)
CoA
Citric acid (initial reactant)
$NADH + H^+$
NAD^+
Malic acid
Isocitric acid
Krebs cycle
CO_2
NAD^+
$NADH + H^+$
Fumaric acid
α-Ketoglutaric acid
$FADH_2$
CoA
CO_2
NAD^+
FAD
Succinic acid
Succinyl-CoA
$NADH + H^+$
CoA
GTP
GDP + P_i
ADP
ATP
Key:
P_i = Inorganic phosphate
CoA = Coenzyme A

Fig. 4.6: Pathways of the TCA cycle

Ans: Alpha-ketoglutaric aciduria and fumarase deficiency are disorders of TCA cycle. These disorders are associated with symptoms such as developmental delay, hypotonia, spasticity, and variable extrapyramidal symptoms (movement disorders).

Hexose Monophosphate Shunt (Pentose Phosphate Pathways)

SAQ: What is the significance of pentose phosphate pathways (hexose monophosphate shunt)?

Ans: The pentose phosphate pathway (hexose monophosphate shunt) is a process that generates NADPH and pentoses (5-carbon sugars). The first phase of these pathways is the reversible oxidative phase, in which NADPH is generated. It is used for the synthesis of fatty acids and steroids. In the second phase, non-oxidative synthesis of 5-carbon sugars takes place. Ribose is used for nucleotide and nucleic acid formation. The second phase is reversible, in which ribulose 5-phosphate is converted back to glucose 6-phosphate using mainly two enzymes: Transketolase and transaldolase.

LAQ: Describe the pentose phosphate pathways (hexose monophosphate shunt).

Ans: The pentose phosphate pathways (hexose monophosphate shunt) take place in the cytosol of a cell. It is a process that generates NADPH and pentoses (5-carbon sugars). It takes place in two phases.

The first phase of these pathways is the reversible oxidative phase, in which NADPH is generated. It is used for the synthesis of fatty acids and steroids.

In the second phase, non-oxidative synthesis of 5-carbon sugars takes place. Ribose is used for nucleotide and nucleic acid formation. The second phase is reversible, in which ribulose 5-phosphate is converted back to glucose 6-phosphate.

Oxidative phase

The following are the various reactions of the oxidative phase:

1. Glucose 6-phosphate catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconate.
2. Phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate, to yield the ketose, ribulose-5-phosphate. $NADP^+$ serves as an oxidant.
3. Reduction of $NADP^+$ involves the transfer of 2 e- and 1 H^+ to the nicotinamide moiety to form NADPH. It is a product of the pentose phosphate pathway and functions as a reductant in anabolic (synthetic) pathways, e.g. fatty acid synthesis.

Regulation of glucose 6-phosphate dehydrogenase

The presence of glucose 6-phosphate dehydrogenase is the committed step of the pentose phosphate pathway. This enzyme is regulated by the availability of the substrate $NADP^+$. As NADPH is utilized in reductive synthetic pathways, the increasing concentration of $NADP^+$ stimulates the pentose phosphate pathway to get back NADPH.

Non-oxidative phase

The rest of the pathway converts ribulose 5-P to the 5-C product ribose 5-P, or to 3-C glyceraldehyde, 3-P and 6-C fructose, 6-P. Additional enzymes required to carry out these reactions include isomerase, epimerase, transketolase, and transaldolase.

Non-oxidative phase reactions take place as follows:

1. Epimerase inter-converts stereoisomers ribulose 5-P and xylulose 5-P.
2. Isomerase converts the ketose ribulose 5-P to the aldose ribose 5-P.

   Both reactions are reversible.

3. Transketolase transfers a 2-C fragment from xylulose 5-P to either ribose 5-P or erythrose 4-P.
4. Transketolase utilizes as prosthetic group thiamine pyrophosphate (TPP), a derivative of vitamin B_1.
   Pyruvate dehydrogenase of Krebs Cycle also utilizes TPP as a prosthetic group.
5. The 3-C aldose glyceraldehyde 3-P is released. A 2-C fragment remains on TPP.
6. The 2-C fragment condenses with one of the aldoses, erythrose 4-P (4-C) or ribose 5-P (5-C) to form a ketose-P product.
7. Transfer of the 2-C fragment to the 5-C aldose ribose 5-phosphate yields sedoheptulose 7-phosphate.
8. Transfer of the 2-C fragment instead to the 4-C aldose erythrose 4-phosphate yields fructose 6-phosphate.
9. Transaldolase catalyzes the transfer of a 3-C dihydroxyacetone moiety from the ketose sedoheptulose 7-phosphate to the aldose glyceraldehydes 3-phosphate to form the ketose fructose 6-phosphate and the four carbon aldose erythrose 4-phosphate.
10. Transketolase catalyzes the reaction in which xylose 5-phosphate serves as a donor of glyceraldehyde, which is accepted by erythrose 4-phosphate to form fructose 6-phosphate and glyceraldehyde 3-phosphate.
    - To oxidize glucose completely to CO_2 via the pentose phosphate pathway, it is necessary that the enzyme be present in the tissue to convert glyceraldehydes 3-phosphate to glucose 6-phosphate.
    - Summarization of flow of 15-C atoms through pentose phosphate pathway reactions by which 5-C sugars are converted to 3-C and 6-C sugars as follows (Fig. 4.7):
      $C_5 + C_5 \rightarrow C_3 + C_7$ (Transketolase)
      $C_3 + C_7 \rightarrow C_6 + C_4$ (Transaldolase)
      $C_5 + C_4 \rightarrow C_6 + C_3$ (Transketolase)
      $3\ C_5 \rightarrow 2\ C_6 + C_3$ (Overall)

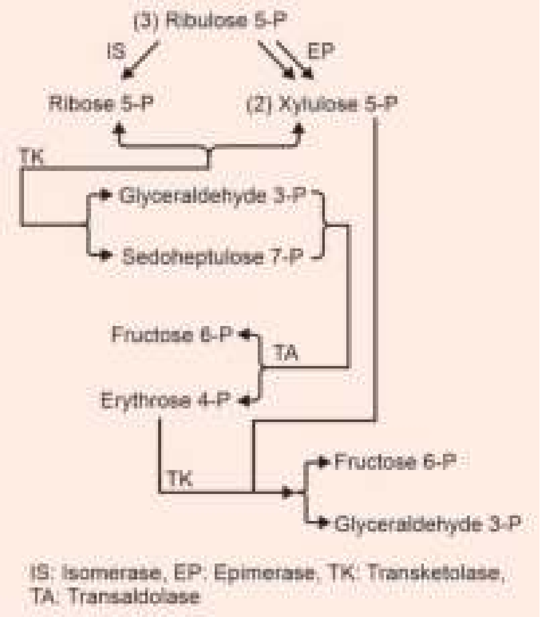

(3) Ribulose 5-P
IS
EP
Ribose 5-P
(2) Xylulose 5-P
TK
Glyceraldehyde 3-P
Sedoheptulose 7-P
Fructose 6-P
TA
Erythrose 4-P
TK
Fructose 6-P
Glyceraldehyde 3-P
IS: Isomerase, EP: Epimerase, TK: Transketolase, TA: Transaldolase

Fig. 4.7: Flow of 15-C atoms through pentose phosphate pathway reactions

NOTE

1. Glyceraldehyde 3-P and fructose 6-P may be converted to glucose 6-P for re-entry to the linear portion of the pentose phosphate pathway, maximizing the formation of NADPH (Fig. 4.8).

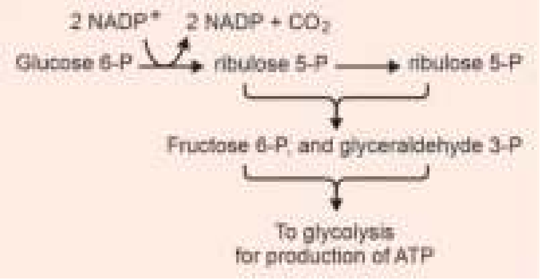

2 NADP⁺
2 NADP + CO₂
Glucose 6-P
ribulose 5-P
ribulose 5-P
Fructose 6-P and glyceraldehyde 3-P
To glycolysis for production of ATP

Fig. 4.8: NADPH generation in HMS pathways

2. Ribose 1-phosphate generated during the catabolism of nucleosides also enters glycolysis in this way after first being converted to ribose-5-phosphate. Thus the pentose phosphate pathway serves as an entry into glycolysis for both 5-carbon and 6-carbon sugars.

SAQ: Why G6PD deficiency leads to hemolytic anemia?

Ans: In erythrocytes, the pentose phosphate pathways provide NADPH for the reduction of oxidized glutathione. Reduced glutathione is necessary for the elimination of toxic hydrogen peroxide produced in the metabolic reactions; the reaction is catalyzed by glutathione peroxidase. A deficiency of G6PD in the erythrocytes leads to a significant decrease in the synthesis of NADPH. As a result, oxidized glutathione is not reduced back, leading to the accumulation of hydrogen peroxide in the cell and, eventually early red blood cell death. Excessive destruction of red blood cells leads to hemolytic anemia.

SAQ: What is glutathione?

Ans: Glutathione is a tripeptide. Its functional group is cysteine thiol. Glutathione peroxidase catalyzes the degradation of organic hydroperoxides by reduction, as two glutathione molecules (represented as GSH) are oxidized to a disulfide.

$$2\,GSH + ROOH \rightarrow GSSG + ROH + H_2O$$

Glutathione peroxidase uses the trace element selenium as a functional group.

SAQ: How regeneration of glutathione takes place?

Ans: Regeneration of reduced glutathione requires NADPH, produced within erythrocytes in the pentose phosphate pathway. Glutathione reductase catalyzes the following reaction to obtain GSH:

$$GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$$

SAQ: What is the clinical significance of genetic deficiency of Glucose 6-P Dehydrogenase (G6PD) in red blood cells?

Ans: Genetic deficiency of glucose 6-P dehydrogenase can lead to hemolytic anemia due to inadequate NADPH within red blood cells. In red blood cells for reduced glutathione, NADPH molecules are required to regenerate back to the oxidized form. Oxidized glutathione then acts on harmful oxidized molecules like hydrogen peroxide produced in oxidative stress (caused due to drugs like antimalarials) to form water and gets reduced in this process. In the inadequate supply of NADPH, excessive destruction of red cells takes place, leading to hemolytic anemia.

The effect of a partial deficiency of glucose 6-phosphate dehydrogenase is exacerbated by substances that lead to increased production of peroxides (e.g. the antimalarial drugs).

Uronic Acid Pathways

SAQ: What is the significance of the uronic acid pathway?

Ans: The substances formed by uronic acid pathways are known as glucuronides. In the body, glucuronic acid is often used to make excretory substances like bilirubin more soluble for excretion by a process known as glucuronidation, which occurs mainly in the liver by the enzyme UDP-glucuronyltransferase.

The human body uses glucuronidation for the subsequent elimination of a variety of excretory substances from the body through urine or feces. Hormones may also be glucuronidated to allow for easier transport around the body. Many toxic substances become less toxic after glucuronidation. Glucuronidation is useful in the excretion of substances such as bilirubin, androgens, estrogens, mineralocorticoids, glucocorticoids, fatty acid derivatives, retinoids, bile acids, drugs, and pollutants.

LAQ: Describe uronic acid pathways and their significance.

Ans: This is the alternate oxidative pathway for glucose metabolism. Glucose is converted to glucuronic acid and pentoses. In humans and some animals, ascorbic acid is not produced. Glucuronic acid is common in carbohydrate chains of proteoglycans. It is a part of mucous, animal secretions such as saliva, cell glycocalyx, and intercellular matrix (e.g. hyaluronic acid).

In the body, glucuronic acid is often used in the xenobiotic metabolism of substances (glucuronidation) such as bilirubin, androgens, estrogens, mineralocorticoids, glucocorticoids, fatty acid derivatives, retinoids, bile acids, drugs, and pollutants. Glucuronidation occurs mainly in the liver by the enzyme UDP-glucuronyltransferase.

The substances resulting from glucuronidation are known as glucuronides and are much more water-soluble than the non-glucuronic acid-containing substance from which they were originally synthesized. The human body uses glucuronidation to make a large variety of substances more water-soluble and, in this way, allow for their subsequent elimination from the body through urine or feces (via bile from the liver). Hormones may also be glucuronidated to allow for easier transport around the body. Many toxic substances become less toxic after glucuronidation. Following are various steps of the uronic pathway:

1. Glucose 6-P is converted to glucose 1-P by phosphoglucomutase, which then reacts with UTP to form UDP-glucose. The enzyme UDP-glucose pyrophosphorylase catalyzes this reaction.
2. UDP-glucose is oxidized by UDP-glucose dehydrogenase in the presence of NAD to form UDP-glucuronic acid, which is the "active" form of glucuronic acid.
3. Glucuronic acid (glucuronate) is reduced to L-gulonic acid in the presence of NADPH. L-gulonic acid is precursor of ascorbic acid.

NOTE

The enzyme L-gluconolactone oxidase is absent in man (other primates and guinea pigs), which converts gluconate to ascorbic acid (vitamin C). Hence vitamin C should be supplemented in the diet of man (and other primates and guinea pigs).

1. In man, L-gulonic acid is oxidized to 3-keto-L-gulonic acid, which is then decarboxylated to L-xylulose.
2. L-xylulose is converted to D-xylulose in the presence of NADPH.
3. D-xylulose is converted to D-xylulose-5-P via xylitol (reaction catalyzed by L-xylitol dehydrogenase), and then it enters HMP-shunt (Fig. 4.9).

SAQ: What is essential pentosuria?

Ans: Essential pentosuria is a rare hereditary disease. Large quantities of L-xylulose appear in the urine due to the absence of L-xylitol dehydrogenase, preventing the reduction of L-xylulose to xylitol. Administration of drugs such as antipyrine and aminopyrine (analgesic and antipyretic), increases the excretion of L-xylulose in pentosuric patients. Essential pentosuria is asymptomatic without any ill effects.

SAQ: Name two drugs that increase glucuronic acid.

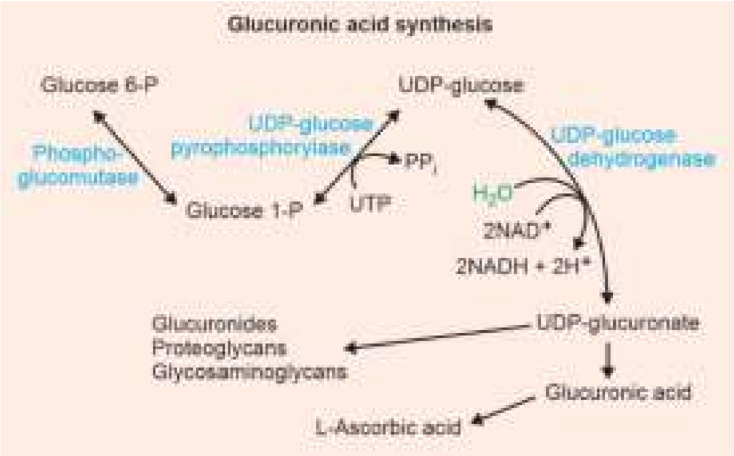

Glucuronic acid synthesis
Glucose 6-P
Phospho-glucomutase
UDP-glucose pyrophosphorylase
PPi
Glucose 1-P
UTP
UDP-glucose
UDP-glucose dehydrogenase
H2O
2NAD+
2NADH + 2H+
UDP-glucuronate
Glucuronides
Proteoglycans
Glycosaminoglycans
Glucuronic acid
L-Ascorbic acid

Fig. 4.9: Uronic acid pathways

Ans: Administration of drugs such as chlorobutanol and barbitone (barbital) significantly increases the synthesis of glucuronic acid by stimulating "uronic acid" pathways.

BAQ: Enumerate functions of glucuronic acid in metabolic pathways.

Ans: The following are the various functions of glucuronic acid:

1. D-glucuronic acid combines with benzoic acid to form benzoyl glucuronide, and phenol combines with D-glucuronic acid to form phenylglucuronide. These intermediate compounds are formed in the liver.
2. Bilirubin conjugates with D-glucuronic acid to form mono and di-glucuronides in the liver and is then excreted through bile.
3. Drugs and other xenobiotics are first hydroxylated by mono-oxygenase cytochrome P-450 system and then conjugated with D-glucuronic acid. Certain antibiotics like chloramphenicol conjugate with D-glucuronic acid.
4. Hormones like thyroxine and derivatives of steroid hormones are detoxified by D-glucuronic acid.
5. D-glucuronic acid is incorporated into hyaluronic acid, chondroitin sulfate (an important structural component of cartilage), and heparin.
6. UDP-glucuronic acid is changed to L-iduronic acid and incorporated into dermatan sulfate (a monosaccharide found in the skin).
7. UDP-glucuronic acid is decarboxylated in cornea and cartilage by a specific enzyme and NAD to form UDP-xylose, which is used in the synthesis of mucoproteins.

GLUCONEOGENESIS

SAQ: What is the significance of gluconeogenesis?

Ans: Gluconeogenesis is a metabolic pathway that results in the generation of glucose molecules from noncarbohydrate carbon substrates such as glycerol, lactate, and glucogenic amino acids. It is one of the two main mechanisms the body uses to prevent hypoglycemia, mainly during the fasting phase.

LAQ: Describe the pathways of gluconeogenesis.

Ans: Gluconeogenesis is a metabolic pathway that results in the generation of glucose molecules from noncarbohydrate carbon substrates such as glycerol, lactate, and glucogenic amino acids.

Gluconeogenesis is a pathway consisting of eleven enzyme-catalyzed reactions. The pathway begins in the mitochondria or cytoplasm, depending on the substrate being used. Many of the following reactions are the reversible steps found in glycolysis:

1. Gluconeogenesis in the mitochondria: In the first step formation of oxaloacetate takes place through the carboxylation of pyruvate. This reaction also requires one molecule of ATP and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in β-oxidation in the liver) and inhibited by high levels of ADP.
2. In the next step, oxaloacetate is reduced to malate using NADH, a step required for the transport of malate out of the mitochondria.
3. Next, malate is oxidized to oxaloacetate using NAD^+ in the cytoplasm, where the remaining steps of gluconeogenesis occur.
4. Oxaloacetate formed is decarboxylated and phosphorylated to produce phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. One molecule of GTP is hydrolyzed to GDP during this reaction (Fig. 4.10A).

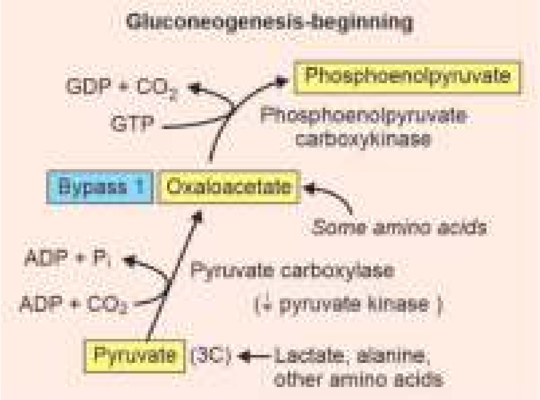

Gluconeogenesis-beginning
GDP + CO$_2$
GTP
Phosphoenolpyruvate
Phosphoenolpyruvate carboxykinase
Bypass 1
Oxaloacetate
Some amino acids
ADP + P$_i$
ADP + CO$_2$
Pyruvate carboxylase
(↓ pyruvate kinase)
Pyruvate
(3C)
Lactate, alanine, other amino acids

Fig. 4.10A: Gluconeogenesis pathways (part-1)

5. Phosphoenolpyruvate is converted to fructose-1, 6-bisphosphate in series of reactions shown in Fig. 4.10B.

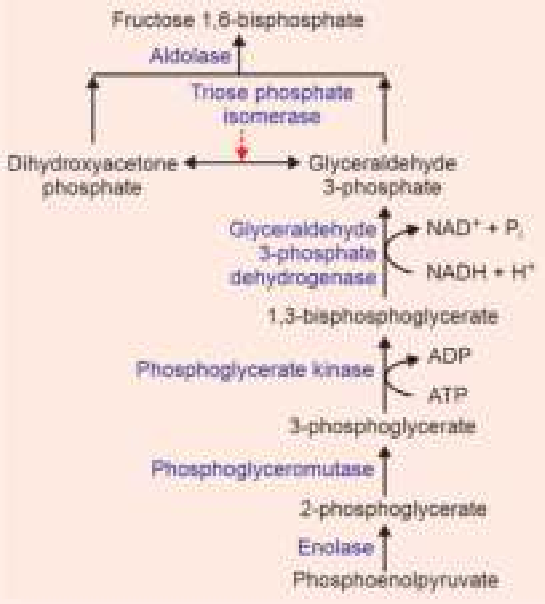

Fructose 1,6-bisphosphate
Aldolase
Triose phosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Glyceraldehyde 3-phosphate dehydrogenase
NAD$^+$ + P$_i$
NADH + H$^+$
1,3-bisphosphoglycerate
Phosphoglycerate kinase
ADP
ATP
3-phosphoglycerate
Phosphoglyceromutase
2-phosphoglycerate
Enolase
Phosphoenolpyruvate

Fig. 4.10B: Gluconeogenesis pathways (part-2)

6. The next steps are the same as reversed glycolysis. However, fructose 1,6-bisphosphatase converts fructose 1,6-bisphosphate to fructose 6-phosphate. This is also the rate-limiting step of gluconeogenesis (Fig. 4.10C).
7. Glucose 6-phosphate is formed from fructose 6-phosphate by phosphoglucoisomerase

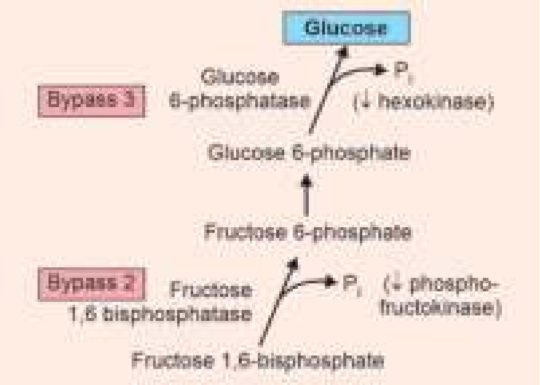

Glucose
Bypass 3
Glucose 6-phosphatase
P$_i$
(↓ hexokinase)
Glucose 6-phosphate
Fructose 6-phosphate
Bypass 2
Fructose 1,6 bisphosphatase
P$_i$
(↓ phosphofructokinase)
Fructose 1,6-bisphosphate

Fig. 4.10C: Gluconeogenesis pathways (part-3)

8. The final reaction of gluconeogenesis, i.e. the formation of glucose, occurs in the lumen of the endoplasmic reticulum where glucose 6-phosphate is hydrolyzed by glucose 6-phosphatase to produce glucose. Glucose is shuttled into the cytosol by glucose transporters located in the membrane of the endoplasmic reticulum.

Note

Gluconeogenesis clears lactate produced by muscle and erythrocytes and also glycerol produced by adipose tissue. Glycerol produced by the lipolysis of triglycerides can be converted into glucose in gluconeogenesis.

All the citric acid cycle intermediates, through conversion to oxaloacetate, can function as substrates for gluconeogenesis. Similarly, amino acids other than lysine or leucine and glycerol can also function as substrates for gluconeogenesis. Deamination or transamination of amino acids facilitates the entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

Fatty acids cannot be converted into glucose in animals, except for odd-chain fatty acids, which yield propionyl-CoA, a precursor for succinyl-CoA. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis.

CORI CYCLE

BAQ: What is the significance of the Cori cycle?

Ans: Lactate formed in anaerobic oxidation of glucose is transported back to the liver, where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase. Pyruvate can then be used to generate glucose by gluconeogenesis (Fig. 4.11).

GLYCOGENESIS

LAQ: Describe biosynthesis and the importance of glycogenesis.

Ans: Glycogenesis is the process of synthesis of glycogen. In this process, glucose molecules are added to chains of glycogen for storage. This process is activated during rest periods following the Cori cycle in the liver. It is also activated by insulin in response to high glucose levels, after a carbohydrate-rich meal.

Glycogen is synthesized depending on the demand for glucose and ATP (energy). If both are present in relatively high amounts, then the excess of insulin promotes glucose conversion into glycogen for storage in liver and muscle cells. Muscle glycogen provides a readily available source of glucose for glycolysis within the muscle cells and liver glycogen functions to store and maintain blood glucose between meals. The highly branched structure of glycogen provides a large number of sites for glycogenolysis for the rapid release of glucose for muscular activities and also to maintain normal blood glucose levels.

The process of glycogenesis takes place as follows (Fig. 4.12):

1. In the first step, glucose is converted into glucose 6-phosphate by the action of hexokinase.
2. In the second step, glucose 6-phosphate is converted into glucose 1-phosphate by the

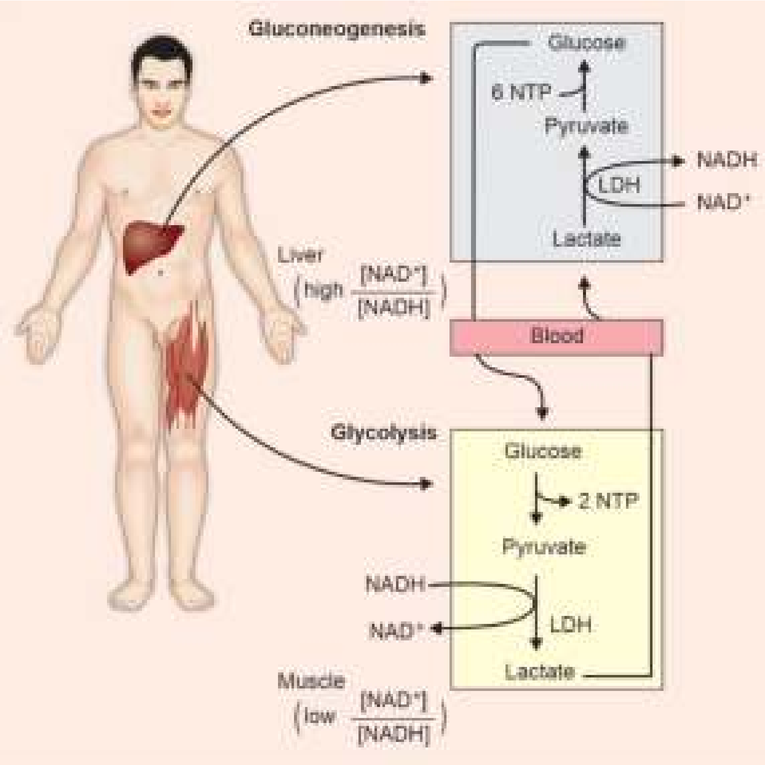


Fig. 4.11: The Cori cycle

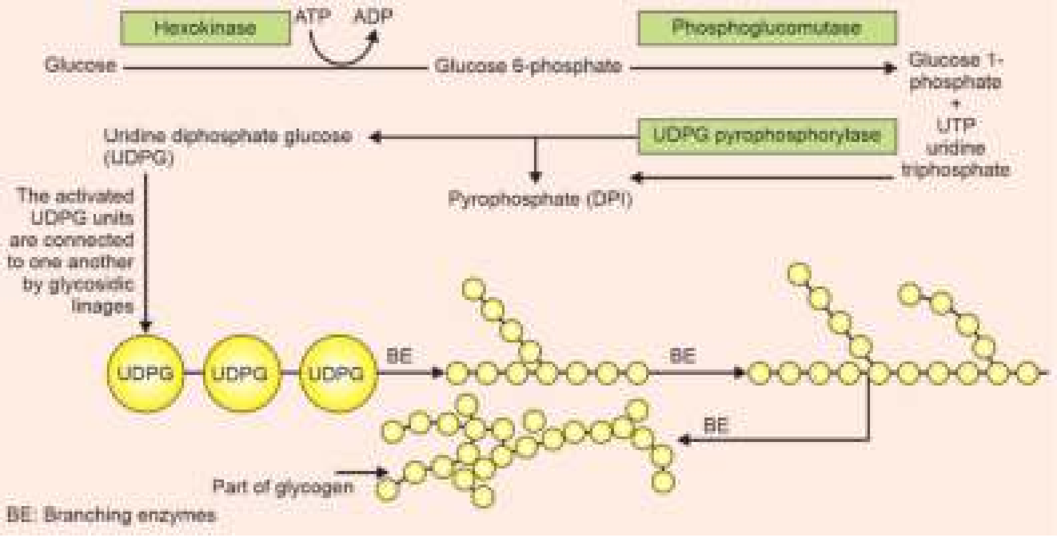

Hexokinase
ATP
ADP
Glucose
Glucose 6-phosphate
Phosphoglucomutase
Glucose 1-phosphate
+
UTP uridine triphosphate
UDPG pyrophosphorylase
Uridine diphosphate glucose (UDPG)
Pyrophosphate (DPI)
The activated UDPG units are connected to one another by glycosidic linages
UDPG
UDPG
UDPG
BE
BE
BE
Part of glycogen
BE: Branching enzymes

Fig. 4.12: Reactions of glycogenesis

action of phosphoglucomutase, passing through an obligatory intermediate step of glucose 1,6-biphosphate.

3. In the next step, glucose 1-phosphate is converted into UDP glucose (UDPG) by the action of uridylyltransferase, and pyrophosphate is formed, which is hydrolyzed by pyrophosphatase into 2 molecules of Pi.
4. Glucose molecules are assembled in a chain by glycogen synthase which must act on a pre-existing glycogen primer or glycogenin (a small protein that forms the primer). The mechanism for joining glucose units is that, glycogen synthase binds to UDPG, causing it to break down into an oxonium ion, also formed in glycogenolysis. This oxonium ion can be readily added to the 4-hydroxyl group of a glucosyl residue on the 4 end of the glycogen chain.
5. Branches are made by branching enzymes (also known as amylo-α(1:4)-α(1:6) transglycosylase) which transfers the end of the chain onto an earlier part via α-1:6 glucosidic bond, forming branches, which further grow by the addition of more α-1:4 glucosidic units.

LAQ: Describe biochemical reactions and the importance of glycogenolysis.

Ans: Glycogenolysis is the catabolism of glycogen by the removal of a glucose monomer through cleavage with inorganic phosphate to produce glucose 1-phosphate. This derivative of glucose is then converted to glucose 6-phosphate, an intermediate in glycolysis. The hormones epinephrine and glucagon stimulate glycogenolysis, which takes place in the muscle and liver tissue, where glycogen is stored. Glycogenolysis takes place as a hormonal response to epinephrine (e.g. adrenergic stimulation) or glucagon (a pancreatic peptide) triggered by low blood glucose concentrations produced in the α-cells of the islets of Langerhans. Glycogenolysis is not the reverse of glycogenesis, but it is a separate pathway. The following reactions take place in the process of glycogenolysis:

1. Glycogen phosphorylase cleaves the α-1,4 glycosidic bonds between the glucosyl residues at the non-reducing ends of the glycogen chains by phosphorolysis. The resultant products are glucose 1-phosphate and the remaining glycogen. Glycogen phosphorylase sequentially

degrades the glycogen chain at their reducing ends.

2. The overall reaction for the 1st step is as follows:
   Glycogen (n residues) + Pi ⟶ Glycogen (n–1 residues) + Glucose 1-phosphate.
3. Branches of glycogen are removed first by glucosyl (4:4) transferase by breaking α-1,4 bonds and α-1,6 bond removed hydrolytically by the amylo-α-1,6-glucosidase activity, releasing free glucose.
4. Glucose 1-phosphate is converted to glucose 6-phosphate by phosphoglucomutase. Hepatic cells can consume the glucose 6-phosphate in glycolysis, or remove the phosphate group using the enzyme glucose 6-phosphatase and release the free glucose into the bloodstream for uptake by other cells. Muscle cells in humans do not possess glucose 6-phosphatase and hence will not release glucose but instead use the glucose 6-phosphate in glycolysis.

SAQ: What is the role of cyclic AMP in glycogenolysis?

Ans: Cyclic AMP coordinates the regulation of glycogenolysis and glycogenesis by promoting the simultaneous activation of phosphorylase and inhibition of glycogen synthase. Insulin acts by inhibiting glycogenolysis and stimulating glycogenesis.

SAQ: What is the role of epinephrine and insulin in glycogenolysis?

Ans: Epinephrine not only activates glycogen phosphorylase but also inhibits glycogen synthase. This amplifies the effect of activating glycogen phosphorylase. Insulin has an antagonistic effect on epinephrine, that does not favor glycogenolysis. Insulin favors glycogenesis.

SMQ: What are glycogen storage diseases? Name 5 glycogen storage diseases.

Ans: "Glycogen storage diseases" are inherited disorders characterized by deposition of abnormal quantity or type of glycogen or failure to mobilize glycogen. Various examples of this type of disease are von Gierke's disease, Pompe's disease, Cori's disease, Hers' disease, Tarui's disease, McArdle's syndrome, etc.

Glycogen Storage Diseases

LAQ: What are Glycogen Storage Diseases? Write the clinical significance of each such disease.

Ans: Glycogen storage disease is a group of inherited disorders characterized by the deposition of abnormal quantity or type of glycogen or failure to mobilize glycogen. Various examples of this type of disease are von Gierke's disease, Pompe's disease, Cori's disease, and Hers' disease. Tarui's disease, McArdle's syndrome, etc.

- von Gierke's disease is caused by the deficiency of glucose 6-phosphatase. Clinical features of this disease are glycogen accumulation in the liver and renal tubules, lactic acidemia, ketosis, hypoglycemia, and hyperlipemia.
- Pompe's disease is caused by the deficiency of lysosomal α-1 → 4 and α-1 → 6 glucosidase. Clinical features of this disease are accumulation of glycogen in lysosomes and muscular dystrophy.
- Forbe's or Cori's disease is caused by the deficiency of branching enzymes. Clinical features of this disease are hepatomegaly in infancy, accumulation of branched polysaccharides, and fasting hypoglycemia.
- Anderson's disease (amylopectinosis) is caused by the deficiency of branching enzymes. Clinical features of this disease are hepatosplenomegaly and death in the first year of life from heart failure or liver failure.
- McArdle's disease is caused by the deficiency of muscle phosphorylase and clinical features of this disease are an increase in muscle glycogen and poor exercise tolerance.

- Hers' disease is caused by the deficiency of liver phosphorylase and clinical features of this disease are the accumulation of glycogen in the liver, mild hypoglycemia, and hepatomegaly. Hers' disease does not cause mental retardation or reduce the life span.
- Tarui's disease is caused by the deficiency of muscle and erythrocyte phosphofructokinase. Clinical features of this disease are hemolytic anemia, high muscle glycogen, poor exercise tolerance, and low levels of lactate after exercise.

SAQ: What is blood sugar? What is the average blood sugar in 24 hours?

Ans: Blood sugar is α-β-D glucose. The concentration of blood glucose remains steady up to 100 mg/dl during the 24 hours, and following food intake; it rises to 140 to 150 mg/dl and returns to about 80 to 90 mg/dl two hours following food.

Competency achievement: The student should be able to:

BI3.5: Describe and discuss the regulation, functions, and integration of carbohydrates along with associated diseases and disorders

BLOOD SUGAR (GLUCOSE)

LAQ: Write on the regulation of blood sugar (glucose). How blood glucose is maintained at normal limits in 24 hours.

Glucose is added to the blood in the following ways:

1. Absorption from the intestine
2. By glycogenolysis
3. By gluconeogenesis

From the blood circulation, glucose is reduced by:

1. Conversion to liver glycogen
2. Conversion to tissue glycogen
3. By synthesis of fats (lipogenesis)
4. Synthesis of lactose and glycoproteins, etc.

A balance of these processes maintains blood sugar levels at normal limits throughout the day with the controlling influence of insulin (hypoglycemic effect) and other hormones (glucagon, epinephrine, norepinephrine, thyroxine, etc. hyperglycemic effect). The maintenance of stable levels of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms. A steady blood glucose level is significant for the nourishment of the individual tissues. Particularly, functions of the brain are dependent on normal blood glucose levels since it has no reserve of oxidizable carbohydrates.

A balance of these various processes keeps the blood sugar level within normal limits (70–110 mg/dl) throughout the day (average 100 mg/dl).

Following organs play a very important role in the maintenance of blood sugar:

1. Liver plays an important role by the developed mechanism of (1) Uptake of glucose from the blood, (2) Conversion of glucose to glycogen, (3) Release of glucose from glycogen, and (4) Conversion of pyruvate to glucose.
2. Muscle glycogen does not contribute directly to blood sugar. Glycogenolysis in muscle produces lactate, which is converted to glucose in the liver.
3. Kidneys exert a regulatory effect by reabsorption of glucose by the tubules completely when the blood glucose level is below 150–170 mg/dl (threshold level). In the case of normal individuals, blood glucose level does not rise above their threshold level. It is due to the role of various hormones.

HORMONAL REGULATION OF BLOOD GLUCOSE

BAQ: Enumerate names of hormones that play important roles in glucose metabolism and their specific function.

Ans: Insulin, glucagon, epinephrine, norepinephrine, and thyroxine play important roles in the regulation of blood glucose. During the fed state, insulin is responsible for the cellular uptake and metabolism of glucose for ATP production and also for glycogenesis. The action of insulin is hypoglycemic.

During the fasting state, glucagon, epinephrine, norepinephrine, and thyroxine maintain normal levels of blood glucose by their hyperglycemic action by facilitating glycogenolysis and gluconeogenesis.

The maintenance of stable levels of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms. A steady blood glucose level is significant for the nourishment of the individual tissues. Particularly, functions of the brain and red blood cells are dependent on normal blood glucose levels since these have no reserve of oxidizable carbohydrates.

BAQ: What are the functions of insulin in glucose metabolism?

Ans: Once in the blood, glucose travels throughout the body. It comes in contact with all cells, including the pancreatic beta cells. These cells make and secrete insulin in response to increased glucose in the blood. Insulin then travels through the blood and attaches itself to insulin receptors on each cell surface. Only then do glucose molecules enter the cells to be used for energy. The cells require a small part of the energy as heat energy. Most of the energy is stored in high-energy bonds such as adenosine triphosphate (ATP). The cell can use this for muscle contraction, electrical energy of nerve impulses, synthesis of new compounds, etc. Extra glucose can be taken up by the liver cells and changed to the storage form glycogen. In muscles also, the extra glucose can be stored as glycogen.

BAQ: What are the normal ranges (reference ranges) of fasting and post-prandial (PP) blood glucose?

Ans: In the case of a normal individual, the fasting blood glucose level is 70–110 mg/dl. Following food intake, it may rise to 140 to 150 mg/dl within one hour, and then after two hours, due to the action of insulin, it drops to the normal level (70–110 mg/dl). Afterward, it does not fall below 70 mg/dl, due to the antagonizing effect of other hormones such as thyroxine, epinephrine, glucagon, etc.

BAQ: What is the role of kidneys in the maintenance of normal blood glucose?

Ans: When blood sugar rises to relatively high levels during the fed state, the kidney also exerts a regulatory effect. Glucose is continuously filtered by the glomeruli but returns to blood by the reabsorption system of the renal tubules.

SAQ: Enumerate the functions of the liver in glucose metabolism.

Ans: Liver plays an important role by the developed mechanism of: (1) Uptake of glucose from the blood, (2) Conversion of glucose to glycogen, (3) Release of glucose from glycogen, and (4) Conversion of pyruvate to glucose.

SAQ: Enumerate the functions of muscles in glucose metabolism.

Ans:

1. Muscles oxidize glucose and get ATP molecules of cellular activities.
2. Excess glucose is stored in muscles in the form of glycogen. In a fasting state, by glycogenolysis, glucose is available for cells.
3. Similarly, lactic acid produced in the anaerobic oxidation of glucose is passed to the liver for conversion to glucose by gluconeogenesis.

SAQ: What is the normal renal threshold of blood glucose? In which clinical condition normal renal threshold of blood glucose is crossed? What is glycosuria?

Ans: Normal renal threshold of blood glucose is 150–170 mg/dl. In hyperglycemia mainly in uncontrolled diabetes mellitus, when blood glucose exceeds 150–170 mg/dl, renal threshold is crossed by blood glucose level and glucose appears in urine. The presence of glucose in the urine is called glycosuria.

LAQ: Write a note on insulin.

Ans: Insulin is a protein hormone produced by the β cells of the islets of Langerhans in the pancreas. Human insulin consists of 51 amino acids in A and B chains joined by two disulfide bridges, with a third disulfide bridge within the A chain (molecular weight: 5808) (Fig. 4.13).

During the synthesis of insulin, first pre-proinsulin (protein of about 100 amino acids, molecular weight 12,000) is formed in the rough endoplasmic reticulum of the β cells of the pancreas. It is rapidly converted to proinsulin (molecular weight 9000) by cleaving enzymes. Proinsulin is stored in the Golgi complex of β cells. Proteolytic cleavage of proinsulin takes place by the release of insulin and C-peptide, in response to glucose, amino acids, gastrointestinal hormones, and certain drugs (e.g. sulfonylureas).

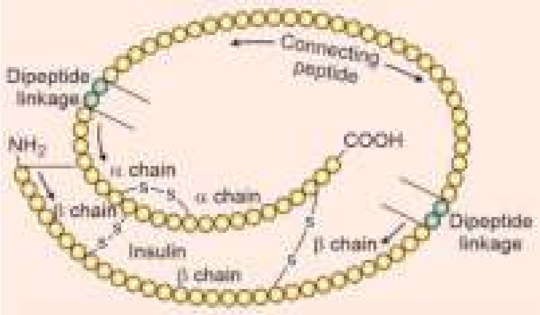


Fig. 4.13: Insulin molecule

Insulin is a hypoglycemic hormone secreted by β cells of the pancreas in response to hyperglycemia. It functions in the following ways to control blood sugar:

1. Increasing the transport of glucose across the cell membrane.
2. Promoting glycolysis.
3. Increasing glycogenesis and
4. Decreasing glycogenolysis.

Insulin also promotes the conversion of acetyl-CoA to fatty acids, thus promoting the synthesis and deposition of fat. It inhibits gluconeogenesis from protein and favors the synthesis of protein from amino acids. Insulin also promotes the transfer of potassium, phosphate, and amino acids into the cells.

Various other substances such as free fatty acids, ketone bodies and drugs such as tolbutamide, sulfonylurea, and hormones like glucagon and secretin also cause the release of insulin. Epinephrine and norepinephrine, however, block the release of insulin.

The beta cells of the pancreas are capable of measuring the blood glucose level, and as the blood glucose level rises, insulin is released in a pulsative fashion in two stages. The first stage occurs within 1–2 minutes, during which insulin molecules are released from the proinsulin molecules stored in secretory granules of the cells by cleavage of proinsulin molecules and with the release of C-peptide. With the rise in blood sugar level, during the second stage, more amount of insulin is manufactured by the beta cells of the pancreas and the amount of insulin released depends upon the rise in blood glucose concentration. The second phase lasts until glucose levels drop to normal levels in 90 to 120 minutes.

Insulin release into the blood begins before the rise in blood glucose level. Food entering the digestive tract stimulates the release of insulin from the pancreatic beta cells. This release depends on the type and amount of food eaten. Sugars and starches are the most effective stimulators. Proteins also help insulin secretion. The combined effects of hormones from the digestive tract and increasing blood glucose levels stimulate the release and formation of insulin. A normal individual can normally manufacture and release 40 to 50 units of insulin, which is only one-fifth of that stored in his pancreas. Thus insulin reserve is plentiful, and it is secreted in exactly the correct amount.

The released insulin molecules bind to the receptors on the plasma membrane of peripheral tissues and adipose tissue. The number of hexose carriers increases in number by allowing the glucose molecules to enter the cells for further metabolism. The ability of insulin to facilitate the transport of glucose inside the cells thus increases all pathways of glucose metabolism, including glycogen deposition, and stimulation of HMP shunt pathways, resulting in increased production of NADPH and increased glycolysis, resulting in increased production of ATP. In adipose tissues, insulin increases lipid synthesis by providing acetyl-CoA and NADPH required for fatty acid synthesis and also glycerol moiety for triglyceride synthesis.

Insulin also exhibits various other activities, including RNA synthesis at the nuclear site, translation at the ribosomal level for protein synthesis, and an influence on the tissue levels of cyclic AMP. Insulin is most active in skeletal and heart muscle, adipose tissue, liver, leukocytes, and the lens of the eye.

Degradation of insulin and C-peptide

In the first circulation through the liver, approximately 50% of insulin is degraded. The remaining insulin is passed through the kidneys, filtered at the glomeruli, reabsorbed, and degraded in the proximal tubules. The half-life (the period taken for the amount of insulin undergoing decrease by half) of insulin in blood circulation is 4–5 minutes.

C-peptide is not extracted by the liver. It passes through the kidneys and is degraded and some part of it is excreted in urine unchanged. The half-life of C-peptide is about 35 minutes. Fasting plasma C-peptide concentration is 5–10 times higher than fasting plasma insulin concentration.

LAQ: Write a note on hormonal regulation of normal glucose metabolism.

Ans: Insulin, glucagon, glucocorticoids, thyroxine, and somatostatin are responsible to maintain normal glucose metabolism. Their respective functions are as follows:

Insulin

Insulin is a hypoglycemic hormone secreted by β-cells of the pancreas in response to hyperglycemia. It functions in the following ways to control blood sugar:

1. Increasing the transport of glucose across the cell membrane
2. Promoting glycolysis
3. Increasing glycogenesis and
4. Decreasing glycogenolysis

Insulin also promotes the conversion of acetyl-CoA to fatty acids, thus promoting the synthesis and deposition of fat. It inhibits gluconeogenesis from protein and favors the synthesis of protein from amino acids. Insulin also promotes the transfer of potassium, phosphate, and amino acids into the cells.

Various other substances such as free fatty acids, ketone bodies and drugs such as tolbutamide, sulfonylurea, and hormones like glucagon and secretin also cause the release of insulin. Epinephrine and norepinephrine, however, block the release of insulin.

Glucagon

Glucagon is produced by the alpha cells of the islets of Langerhans of the pancreas. Its secretion is stimulated by hypoglycemia. It causes glycogenolysis in the liver by activating phosphorylase. Glucagon also enhances gluconeogenesis from amino acids.

Epinephrine

Epinephrine is the hormone secreted by the adrenal medulla. It stimulates glycogenolysis in both the liver and muscle. It activates the enzyme phosphorylase and increases the production of cyclic AMP. Secretion of epinephrine is stimulated during hypoglycemia, fear, anxiety, and anger. Under these conditions, blood sugar level remains elevated. Epinephrine also diminishes the uptake of glucose by tissue cells.

Glucocorticoids

Glucocorticoids, corticosterone, 11-dihydrocorticosterone, 11-dihydro–17-hydroxy corticosterone (cortisone), and 17-hydroxy corticosterone (cortisol) are secreted by the adrenal cortex and known as 11-oxycorticoids. These hormones stimulate gluconeogenesis in the liver mainly from amino acids. Glucocorticoids inhibit the utilization of glucose in extrahepatic tissue. In all these actions, glucocorticoids act in an antagonistic manner to insulin.

Hormones of the anterior pituitary gland

Hormones of the anterior pituitary gland secrete hormones that tend to antagonize the action of insulin, causing a rise in blood glucose. These hormones are growth hormone (GH) and adrenocorticotropic hormone (ACTH) mainly. Growth hormone decreases glucose uptake in muscle. The effect of ACTH on carbohydrate metabolism is due to its stimulation of the secretion of hormones of the adrenal cortex.

Thyroxine

Thyroxine is secreted by the thyroid gland, and it stimulates glycogenolysis and increases the rate of both gastric emptying and intestinal glucose absorption. In hyperthyroid patients, the fasting blood glucose level is elevated, and it is decreased in hypothyroid patients.

Somatostatin

Somatostatin is a polypeptide found mainly in the hypothalamus and the delta cells of the pancreatic islets. It inhibits the release of growth hormone by the pituitary. Somatostatin also inhibits the secretion of glucagon and insulin by the pancreas.

BAQ: Describe renal glycosuria under the following heads:

1. Biochemical basis
2. Change in glucose excretion
3. Treatment

Ans:

1. Normal renal threshold for blood glucose is 150–170 mg/dl. A detectable amount of glucose is not present in the case of a normal individual, since the renal threshold for blood glucose is not crossed. However, there are some individuals, whose renal threshold may be lower than the normal renal threshold for blood glucose. It may be 130–150 mg/dl. This is a case of lowered renal threshold for glucose. In this case, glucose may be detected in post-prandial urine.
2. In the case of a lowered renal threshold for glucose, when blood sugar crosses the 130–150 mg/dl range, glucose excretion takes place, 2 hours following food.
3. No treatment, since the person with lowered renal threshold is a normal individual.

DERANGED GLUCOSE METABOLISM

SAQ: What is deranged glucose metabolism?

Ans: Deranged glucose metabolism means disturbed glucose metabolism mainly due to deficiency of the hormone, insulin as seen in the disease diabetes mellitus. A state of hyperglycemia is produced as a result of a deficiency of available and effective insulin. This lack can be absolute when the pancreas does not produce sufficient insulin or relative when the pancreas produces a normal amount of insulin, but due to insulin resistance the body cells are unable to use glucose and the patient suffers from hyperglycemia.

LAQ: Describe various metabolic changes that take place in uncontrolled diabetes mellitus.

Ans: The following are the various metabolic changes in the body of a diabetic person that occur due to a deficiency of insulin, leading to deranged glucose metabolism:

1. The state of hyperglycemia occurs as a result of impaired transport and uptake of glucose into muscle and adipose tissue.

2. Repression of the glycolytic enzymes and stimulation of gluconeogenic enzymes promote gluconeogenesis in the liver, which further contributes to hyperglycemia.
3. Transport and uptake of amino acids are also depressed in peripheral tissue. This causes an increase in the circulating amino acids, which provide the precursors for gluconeogenesis in the liver. The breakdown of amino acids results in increased production of urea (pre-renal condition).

$$\begin{tikzcd}
\text{Amino acids} \arrow[r, "\text{Deamination}"] & \text{Keto acids} \arrow[d] & + & NH_3 \arrow[d] \\
& \text{Citric acid} \arrow[d] & & \text{Urea cycle} \arrow[d] \\
& CO_2 + H_2O + ATP & & \text{Excreted by kidneys}
\end{tikzcd}$$

4. Due to impaired uptake of glucose by various cells, there is decreased production of ATP, NADPH, and glycerophosphate, and hence a decrease in fatty acid and lipid synthesis.
5. To meet energy requirements, stored lipids are hydrolyzed by increased lipolysis, and the liberated fatty acids then interfere at several steps of carbohydrate phosphorylation in muscle and then further contribute to hyperglycemia.
6. Excessive catabolism of fatty acids gives rise to increased formation of acetyl-CoA, which further contributes towards gluconeogenesis causing hyperglycemia. The high levels of fatty acids inhibit the citric acid cycle at the level of citrate synthetase. The acetyl-CoA, which cannot enter the citric acid cycle or is used for fatty acid synthesis, is shunted to the synthesis of ketone bodies, causing the condition ketosis, which may further lead to acidosis. If this condition is not treated, then it may eventually lead to diabetic coma. Excessive cholesterol formation also may take place from acetyl-CoA, leading to the condition of atherosclerosis.

$$\begin{tikzcd}
& \text{May lead to atherosclerosis} & \\
& \text{Cholesterol} \arrow[u] & \\
\text{Fatty acids} \arrow[r] & \text{Acetyl-COA} \arrow[u] \arrow[r] \arrow[d] & \text{Citric acid cycle} \arrow[d] \\
& \text{Ketone bodies} \arrow[d] & CO_2 + H_2O + ATP \\
& \text{May lead to acidosis and ketosis} &
\end{tikzcd}$$

7. Glycogen synthesis is depressed due to decreased glycogen synthetase activity and by activation of phosphorylase through the action of epinephrine and glucagon and also by increased ADP: ATP ratio.
8. In general, an abnormal balance in inter-hormone control results due to insulin deficiency. A state of hormonal imbalance is produced, which favors the action of corticosteroids, glucagon, and growth hormone. This adds to the stimulation of decreased intracellular metabolism of glucose and favors lipolysis, glycogenolysis and gluconeogenesis.
9. As a result of the unavailability of glucose to the cells, the cells lack fuel, and the body suffers from a lack of energy. People with diabetes complain of weakness and tiredness. When the cells are deprived of their fuel, the body triggers a sense of extreme hunger called polyphagia.
10. Hyperglycemia leads to excess circulation of unused glucose through the kidneys. When the renal threshold for glucose (150–170 mg/dl) is exceeded, glucose escapes into the urine causing glycosuria. The osmotic pressure of the glucose present in urine leads to the excretion of an increased volume of urine. Hence, there is polyuria with the frequency of micturition and pale urine. Up to 5 to 6 liters of urine may be passed daily. The glucose present increases the specific gravity of urine, which may be as high as 1.040. A further consequence of polyuria is increased thirst (polydipsia), which is another characteristic symptom of diabetes. At high glucose concentrations,

the enzyme aldose reductase catalyzes the formation of sorbitol from glucose, and the accumulation of sorbitol may increase the rate of development of cataracts.

DIABETES MELLITUS

BAQ: Describe various symptoms and clinical conditions related to diabetes mellitus.

Ans: The following are the various symptoms and clinical conditions related to diabetes mellitus:

- Lack of energy, weakness, tiredness, loss of weight, and polyuria are the symptoms, due to which the patient probably consults a physician.
- Skin symptoms: Itching of the skin, usually in the genital or anal areas, and frequent appearance of carbuncles and furuncles. Difficulty in healing wounds may also be observed.
- Gynecological problems: Women with hyperglycemia are more likely to get a fungal infection, which causes vaginal itching, and sometimes a chronic discharge may be observed.
- Nerve damage: Numbness, burning, tingling, or intense sensitivity in the skin of feet and legs are the signs of nerve damage. Nerve damage occurs usually after diabetes has been present for a long period.
- Blurred vision: Hyperglycemia may cause glucose to seep into the lens of the eye. The change in the shape of the lens then leads to blurred vision. The vision improves after treatment.

SAQ: Enumerate the characteristic features of diabetes mellitus.

Ans: The characteristic features of diabetes mellitus are:

- Hyperglycemia
- Polyuria
- Thirst
- Loss of weight
- Ketosis
- Acidosis

BAQ: Enumerate the main complications of uncontrolled diabetes mellitus.

Ans: Ketosis, acidosis, atherosclerosis, cardiovascular complications, hyperosmotic coma, retinopathy, neuropathy, and nephropathy.

SAQ: What is syndrome X?

Ans: Syndrome X is a group of clinical conditions and laboratory findings consisting of hyperglycemia, dyslipidemia (high serum cholesterol and triglycerides), hypertension, insulin resistance, and hyperinsulinemia. Individuals with this syndrome are at an increased risk for cardiovascular disease.

SAQ: Classify various types of diabetes mellitus.

Ans: The classification system identifies four types of diabetes mellitus:

Type 1 diabetes mellitus

Type 2 diabetes mellitus

Other specific types: Maturity onset

Types: Due to genetic reasons, infections, drug actions and gestational diabetes (seen during pregnancy in some women).

SAQ: What is gestational diabetes mellitus? .

Ans: Gestational diabetes mellitus develops during gestation. Most women classified with gestational diabetes mellitus have normal glucose homeostasis during the first half of the pregnancy and develop a relative insulin deficiency during the last half of the pregnancy, leading to hyperglycemia. Hyperglycemia resolves in most women after delivery but places them at increased risk of developing type 2 diabetes mellitus later in life.

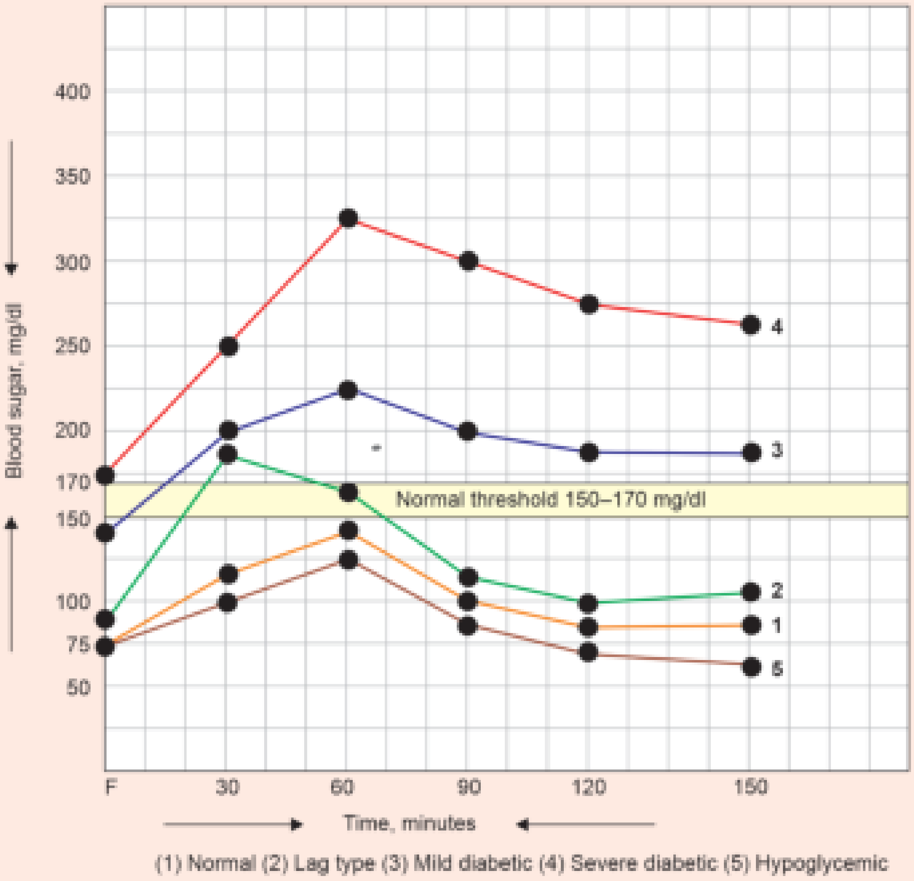
SAQ: What is glucose toxicity?

Ans: Glucose toxicity means a decrease in insulin secretion and an increase in insulin resistance due to chronic hyperglycemia mainly in type 2 diabetes mellitus.

GLUCOSE TOLERANCE TEST

BAQ: Write a short note on glucose tolerance.

Ans: Glucose tolerance means the ability of the body to utilize glucose. It decreases in uncontrolled diabetes mellitus when the person suffers from hyperglycemia, and increases in pancreatic beta cell tumors in which due to hypersecretion of insulin person suffers from hypoglycemia. The status of glucose tolerance can be studied by performing a glucose tolerance test (GTT). In GTT, a fasting blood sample of a patient is collected and after giving him 1.75 g/kg glucose, 4 more blood samples are collected. All collected blood samples are tested for glucose. From the levels of blood glucose values, the status of glucose tolerance could be understood (Fig. 4.14).



Normal glucose tolerance curve						
Time (minutes)	Fasting	30	60	90	120	150
Blood sugar (mg/dl)	75	125	145	100	70	75
Urine sugar	→ Absent throughout ←					

Fig. 4.14: Glucose tolerance test

Evaluation of graphic presentations:

1. Indicates normal glucose tolerance
2. Indicates a moderate decrease in glucose tolerance
3. Indicates a severe increase in glucose tolerance
4. Indicates increased glucose tolerance

BAQ: What is retinopathy and why a diabetic person suffers from retinopathy? What are the symptoms of retinopathy?

Ans: Retinopathy means disease of the retina. In uncontrolled diabetes, due to poor uptake of glucose and narrowing of blood vessels supplying blood to retina, deterioration of retina takes place over time. Similarly, excess blood glucose is converted to sorbitol, which damages the optic nerve. Long-term consequence of uncontrolled diabetes is blindness.

Early symptoms of retinopathy include blur vision, redness seen in the sclera of the eyes, floaters seen in the field of vision, and difficulty in perceiving colours in darkness.

DIABETIC COMPLICATIONS

SAQ: What is neuropathy and why a diabetic person suffers from neuropathy? What are the symptoms of neuropathy?

Ans: Neuropathy means the deterioration of peripheral nerves. In uncontrolled diabetes mellitus, excess blood glucose is converted to sorbitol, which damages the peripheral nerves over time. The symptoms of neuropathy include:

1. Numbness and tingling in the feet or hands
2. Burning, and shooting pain in affected areas
3. Loss of coordination and balance
4. Muscle weakness
5. Burning sensation in the feet and weakness of feet.

BAQ: What is nephropathy and why a diabetic person suffers from nephropathy? What are the symptoms of nephropathy?

Ans: Nephropathy is a deterioration of kidney function. In uncontrolled diabetes, due to the narrowing of blood vessels (atherosclerosis), the blood supply to functioning units of kidneys means to nephrons gets reduced over time. The risk of damage to the basement membrane of the glomerulus also increases, due to chronic microbial infections as a diabetic person becomes immunocompromised. Early symptoms of nephropathy include edema on the face, hand, and feet, the presence of protein in the urine (proteinuria), an increase in blood pressure, and loss of appetite.

BAQ: What is atherosclerosis? Why a diabetic person may suffer from atherosclerosis and cardiovascular disease?

Ans: In uncontrolled diabetes, due to deficiency of insulin or due to insulin resistance body cells are unable to use glucose for energy

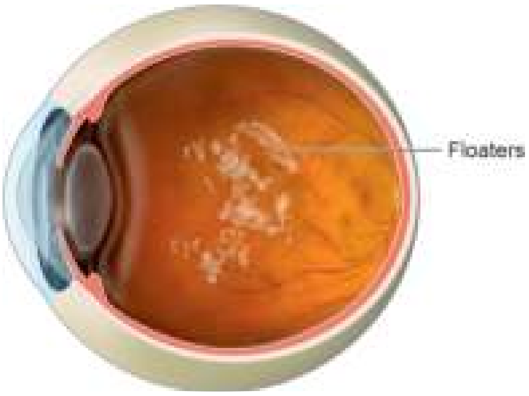


Fig. 4.15: Image of floaters in the eye

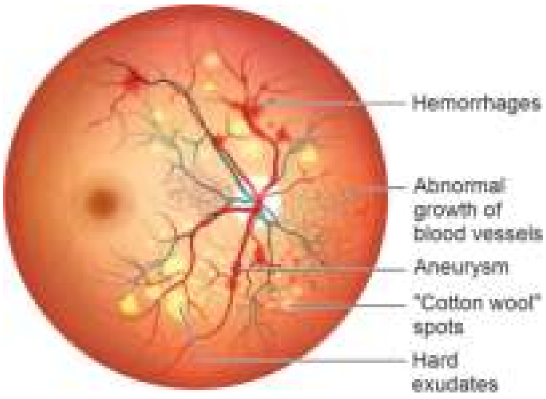


Fig. 4.16: Diabetic retinopathy

(in the form of ATP molecules). That leads to excessive catabolism of fatty acids to get energy. Excessive catabolism of fatty acids gives rise to increased formation of acetyl-CoA, which further contributes towards the excessive synthesis of cholesterol leading to atherosclerosis. Due to increased atherosclerosis, coronary arteries may become narrow, leading to compromised blood supply to cardiac muscles, which may lead to cardiovascular disease.

BAQ: What is acidosis and ketosis? Why an uncontrolled diabetic person may suffer from acidosis and ketosis?

Ans: In uncontrolled diabetes, due to deficiency of insulin or due to insulin resistance body cells are unable to use glucose for energy (in the form of ATP molecules). That leads to excessive catabolism of fatty acids to get energy. Excessive catabolism of fatty acids gives rise to increased formation of acetyl-CoA, which further contributes towards the excessive synthesis of ketone bodies (to derive energy) causing the condition ketosis. Excessive quantity of ketone bodies such as acetone, acetoacetic acid, and beta-hydroxybutyric acid may lead to the clinical condition of acidosis. In acidosis, blood pH drops below the normal level, i.e. below 7.38. If this condition is not treated, then it may eventually lead to diabetic coma.

DIABETIC DRUGS AND TREATMENT

BAQ: What is type I diabetes mellitus? What treatment is given to control type I diabetes mellitus and what precautions are required during the treatment?

Ans: Type I diabetes mellitus is characterized by beta cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency. The onset is usually acute, that develops over a few days to weeks. Over 95 percent of persons with type 1 diabetes mellitus develop the disease before the age of 25, with an equal incidence in both sexes and an increased prevalence in the white population. A family history of type I diabetes mellitus is often seen.

Treatment

In the case of type I diabetes, comprehensive diabetes care is advised by physicians, which involves nutritional care, exercise, regular monitoring of blood glucose, and appropriate administration of insulin since these patients lack endogenous insulin production. Current insulin preparations are generated by using recombinant DNA technology. This type of insulin consists of an amino acid sequence of human insulin. Oral hypoglycemic drugs are not effective in this type of diabetes. Type I diabetic persons undergoing insulin therapy are advised to keep 5–10 g of glucose in their pockets to prevent hypoglycemic attacks, which may arise due to an inappropriate amount of insulin administration, diarrhea, and diet intake.

Q: What is type II diabetes mellitus? What treatment is given to control type II diabetes mellitus and what precautions are required during the treatment?

Ans: Type II diabetes mellitus is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity, and lack of exercise. Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance. Defective beta cells become exhausted, further glucose intolerance and hyperglycemia. The etiology of type II diabetes mellitus is multifactorial.

Treatment

In the case of type II diabetes, the goals of therapy are to maintain normal levels of blood glucose throughout the day and also include normal management of any one of the conditions associated with the patient, such as obesity, dyslipidemia, cardiovascular disease, hypertension, etc. along with an exercise regime. Pharmacologic approaches include oral hypoglycemic drugs in mild diabetes and insulin therapy in moderate or severe type II diabetes.

Type II diabetic persons undergoing therapy are advised to keep 5–10 g of glucose in their pockets to prevent hypoglycemic attacks, which may arise due to an inappropriate amount of hypoglycemic drug administration, diarrhea, and diet.

BAQ: Enumerate various types of oral drugs used to treat Type II diabetes mellitus, with their respective actions.

Ans: Oral glucose-lowering agents are subdivided into agents that increase insulin secretion, reduce glucose production or increase insulin sensitivity.

Sulfonylureas increase insulin secretion. Examples of sulfonylureas are Tolbutamide, Glucotrol (glipizide), Diabinese (Chlorpropamide), Micronase (glyburide), etc.

Biguanides increase insulin sensitivity, increase glucose uptake, and decrease gluconeogenesis. An example of biguanides is metformin.

Thiazolidinediones improve patients' sensitivity to insulin by increasing fatty acid uptake by adipocytes in fat stores. Examples of thiazolidinediones are Rosiglitazone and Pioglitazone.

Alpha-glycoside inhibitors inhibit the enzyme alpha-glycosidase in the small intestine during digestion, which acts on oligosaccharides and release glucose molecules. The action of alpha-glycoside inhibitor leads to delayed glucose absorption and lowering of postprandial blood glucose. Examples of alpha-glucosidase are acarbose (precose) and miglitol (glyset).

Meglitinides stimulate insulin secretion. An example of meglitinide class of drugs is repaglinide.

Dapagliflozin (dopoglifloxin) decreases tubular reabsorption of glucose and facilitates urinary excretion of glucose. Thus, the action of dapagliflozin decreases high glucose levels.

Type II diabetic persons undergoing insulin therapy are advised to keep 5–10 g of glucose in their pockets to prevent hypoglycemic attacks, which may arise due to an inappropriate amount of drug administration.

HYPOGLYCEMIA

BAQ: What is hypoglycemia? What are the symptoms of hypoglycemia? What are the various causes of hypoglycemia and treatment modes?

Ans: Hypoglycemia is a blood glucose concentration below the fasting value (below 50 mg/dl). A transient decline in blood glucose may occur 1.5 to 2 hours after a meal. It is necessary to evaluate hypoglycemia present in the fasting state and that in which the patient has postprandial hypoglycemia (reactive hypoglycemia).

The most common causes of fasting hyperinsulinism, which leads to hypoglycemia, are:

1. Insulinoma,
2. Surreptitious administration of insulin or oral hypoglycemic drug, and
3. The presence of antibodies to insulin.

Clinical symptoms of hypoglycemia fall into two broad categories:

1. Adrenergic and
2. Neuroglycopenic.

Adrenergic symptoms are—weakness, sweating, tachycardia, palpitation, and tremors.

Neuroglycopenic symptoms are—headache, hypothermia, visual disturbances, and mental dullness.

Type II diabetic persons undergoing therapy are advised to keep 5–10 g of glucose in their pockets to prevent hypoglycemic attacks, which may arise due to an inappropriate amount of drug administration.

Patients are treated by giving them about 5.0 g of glucose immediately.

DIAGNOSIS OF DIABETES MELLITUS

BAQ: How diabetes mellitus is diagnosed?

Ans: Diabetes mellitus can be diagnosed by determining fasting and post-prandial (PP) blood and urine glucose. Fasting blood glucose of more than 126 mg/dl and PP blood sugar of more than 200 mg/dl and the presence of glucose in PP urine specimen indicates that the patient is suffering from diabetes mellitus.

BAQ: What are the important diabetic profile tests and their respective importance?

Ans: The following are diabetic profile tests:

1. Blood glucose (F)
2. Blood glucose (PP)
3. Urine glucose (F) and (PP)
4. Urine ketone bodies
5. Blood urea nitrogen reaction
6. Serum total cholesterol
7. Serum triglycerides
8. Glycated (glycosylated) hemoglobin
9. Urine microalbumin

Tests 1, 2 and 3 give an idea about the glycemic control of the patient.

Test 4 indicates ketonuria. With further dehydration (due to polyuria in uncontrolled diabetes) and ketone build-up in the blood, the patient may suffer from acidosis, and in extreme cases or when enough insulin and fluids are not given in time, coma and unconsciousness may occur.

Test 5 values indicate the existence of pre-renal condition due to deranged amino acid metabolism in uncontrolled diabetes mellitus.

Tests 6 and 7 give an idea of the risk of atherosclerosis.

Test 8 gives an idea of long-term glycemic control

Test 9 gives an idea about the status of nephropathy.

BAQ: What is glycosylated hemoglobin (GHb)? What specimen is used for the GHb test? What is the significance of the GHb test?

Ans: The red cells of normal human adults and children above the age of 6 months contain the following three genetically determined hemoglobin fractions: HbA, HbA2, and HbF. These three species comprise 90.0%, 2.5%, and 0.5%, respectively of the total hemoglobin content of red cells. HbA also contains the following three fractions: HbA1a, HbA1b and HbAc which comprise about 1.6%, 0.8%, and 4%, respectively, of the total hemoglobin of red blood cells. HbA1a, HbA1b and HbAc are collectively measured as HbA1.

The rate of formation of HbA1 depends on the life span of red cells and mean glucose concentration of plasma for the past 4–6 weeks. The normal range of HbA1 is 5–8%. Similarly, the normal range of HbA1c is 4–6.5%. The present trend is to determine HbA1c rather than total HbA1; either by manual method or by an automated method.

The specimen used for the blood GHb test is EDTA blood. A fasting sample is not necessary.

Clinical Significance

Blood values of HbA1c (and also HbA1) indicate the average plasma glucose concentration of the previous 4–6 weeks. Blood HbA1c values do not change concerning recent food or glucose intake or diabetic drugs. Blood HbA1c values of more than 6.5% indicate poor glycemic control of the patient. Glycemic control means the maintenance of blood glucose values within normal range by the patient with the use of proper diet and drugs. Patients may manage to bring blood glucose levels within normal range by using an appropriate diabetic drug, however, a physician can find out glycemic control of the patient by blood HbA1c values.

Multiple Choice Questions

Q1. Gluconeogenesis is decreased by the action of which hormone?

A. Insulin B. Glucagon
C. Glucocorticoids D. Thyroxine

Q2. Glucose 6-phosphatase is not present in

A. Kidneys and liver
B. Kidneys and muscles
C. Muscles and adipose tissue
D. Muscles and liver

Q3. Which of the following hormones is responsible for glucose uptake by liver cells?

A. Glucagon B. Insulin
C. Epinephrine D. Norepinephrine

Q4. Renal threshold for glucose is decreased in

A. Diabetes mellitus
B. Renal glycosuria
C. Diabetes insipidus
D. Hepatitis

Q5. Glucose 6-phosphatase is absent or deficient in

A. Cori's disease
B. von Gierke's disease
C. Pompe's disease
D. McArdle's disease

Q6. Debranching enzyme is absent in

A. Hers' disease
B. Andersen's disease
C. von Gierke's disease
D. Cori's disease

Q7. McArdle's disease is due to the deficiency of which of the following enzymes?

A. Phosphofructokinase
B. Muscle myophosphorylase
C. Liver phosphorylase
D. Glucose 6-phosphatase

Q8. In essential pentosuria, urine contains which of the following carbohydrates?

A. D-ribulose B. L-xylulose
C. D-xylose D. D-ribose

Q9. Congenital galactosemia may lead to

A. Mental retardation
B. Premature cataract
C. Death
D. All of the above

Q10. Catalytic activity of salivary amylase requires the presence of

A. Phosphate ions
B. Chloride ions
C. Magnesium ions
D. Calcium ions

Q11. Which of the following monosaccharides is actively absorbed in the intestine

A. Galactose B. Fructose
C. Mannose D. Ribose

Q12. Which of the following is an amphibolic pathway

A. HMP shunt B. Glycolysis
C. Gluconeogenesis D. Citric acid cycle

Q13. In the case of Cori's cycle, there is a transfer of

A. Glucose from muscles to the liver
B. Glucose from liver to muscles
C. Lactate from the liver to muscles
D. Lactate from muscles to the liver

Q14. Ethanol decreases gluconeogenesis by

A. Converting NAD^+ into NADH and decreasing the availability of lactate
B. Converting NAD^+ into NADH and decreasing the availability of pyruvate
C. Inhibiting insulin
D. Inhibiting glycogenesis

Q15. During starvation, ketone bodies are used as fuel by

A. Liver B. Red blood cells
C. Brain D. A, B, and C

Q16. Obesity increases the risk of

A. Diabetes mellitus
B. Gout
C. Cardiovascular disease
D. A and C

Q17. Hexokinase has a higher affinity for glucose than

A. Galactokinase B. Glucokinase
C. Fructokinase D. All of the above

Q18. The carrier compound in the case of the citric acid cycle is

A. Malate B. Fumarate
C. Oxaloacetate D. Succinate

Q19. The conversion of glucogenic amino acids to glucose means

A. Glycolysis B. Gluconeogenesis
C. Glycogenesis D. Glycogenolysis

Q20. The action of epinephrine leads to

A. Glycolysis
B. Glycogenolysis
C. Gluconeogenesis
D. Glycogenesis

Q21. Under anaerobic conditions in the glycolysis one mole of glucose yields

A. Two ATPs
B. One ATP
C. Eight ATPs
D. Thirty-six ATPs

Q22. Excess of carbohydrates in food leads to the formation of the following in the body

A. Carbohydrates
B. Fat
C. Protein
D. Both B and C

Q23. In the body pentoses are obtained from

A. TCA cycle
B. Glycolytic pathway
C. HMP shunt
D. Both A and B

Q24. Conversion of glucose to glucose 6-phosphate in the liver is catalyzed by

A. Hexokinase
B. Glucokinase
C. Glucose 6-PD
D. Both A and B

Q25. Glucose tolerance is increased in

A. Diabetes mellitus
B. Diabetes insipidus
C. Insulinoma
D. Thyrotoxicosis

Q26. Glucose tolerance is decreased in

A. Diabetes mellitus
B. Addison's disease
C. Hypopituitarism
D. Hypothyroidism

Q27. Glycogen is converted to glucose 1-phosphate by

A. G6PD
B. Glycogen phosphorylase
C. Branching enzyme
D. UDPG transferase

Q28. Tricarboxylic acid cycle requires the regeneration of

A. Malic acid
B. Glutamic acid
C. Oxaloacetic acid
D. Pyruvic acid

Q29. Gluconeogenesis is increased in which of the following conditions?

A. Diabetes mellitus
B. Diabetes insipidus
C. Hypothyroidism
D. Insulinoma

Q30. The oxidation of lactic acid to pyruvic acid requires which of the following coenzymes?

A. FMN
B. Coenyzme A
C. NAD^+
D. NADH

Q31. The number of molecules of ATP produced by the total oxidation of acetyl-CoA in the TCA cycle is

A. 4
B. 8
C. 10
D. 2

Q32. Fatty acids cannot be converted into carbohydrates in the body since the following reaction is not possible

A. Formation of acetyl-CoA from fatty acids
B. Transformation of acetyl-CoA to pyruvate
C. Conversion of fructose 1, 6-bisphosphate to fructose 6-phosphate
D. Conversion of glucose 6-phosphate into glucose

Q33. Which of the following are rate-limiting enzymes of gluconeogenesis?

A. Pyruvate carboxylase
B. Phosphoenolpyruvate carboxykinase
C. Fructose 1,6-diphosphatase
D. All of the above

Q34. Fluoride inhibits which of the following enzyme to prevent glycolysis?

A. Succinate dehydrogenase
B. Aconitase
C. Glyceraldehyde 3-phosphate dehydrogenase
D. Enolase

Q35. When the oxygen supply is inadequate, pyruvate is converted to

A. Lactate
B. Acetyl-CoA
C. Alanine
D. Phosphopyruvate

Q36. Activation of liver phosphorylase is normally favored by

A. Glucagon
B. Epinephrine
C. Insulin
D. Thyroxine

Q37. Pyruvic acid is converted to this compound before it enters the TCA cycle

A. Citrate
B. Acetyl-CoA
C. λ-ketoglutarate
D. Lactate

Q38. Which one is the rate-limiting enzyme of gluconeogenesis?

A. Pyruvate kinase
B. Glucose 6-PD
C. Hexokinase
D. Pyruvate carboxylase

Q39. Which animal cannot convert glucose to vitamin C?

A. Cow B. Dog
C. Monkey D. Albino rat

Q40. Gluconeogenesis is increased in

A. Diabetes insipidus
B. Hyperthyroidism
C. Starvation
D. Liver disease

Q41. Pyruvate kinase requires which of these metallic ions for the maximum activity?

A. Mg^{2+} B. K^+ C. Ca^{2+} D. Na^+

Q42. The enzymes involved in the phosphorylation of glucose to glucose 6-phosphate are

A. Hexokinase
B. G6PD
C. Phosphofructokinase
D. Isomerase

Q43. During the normal resting state, most of the blood glucose is used by

A. Kidneys B. Brain
C. Liver D. Adipose tissue

Q44. The following metabolic abnormalities occur in uncontrolled diabetes mellitus, *except*

A. Ketosis
B. Increased deposition of fats in the liver
C. Increased plasma FFA
D. Increased glycolysis

Q45. Which compound is essential for converting glucose to glycogen in the liver?

A. UTP
B. GTP
C. Lactic acid
D. CTP

Q46. The allosteric enzyme responsible for controlling the rate of the TCA cycle is

A. Fumarase
B. Malate dehydrogenase
C. Isocitrate dehydrogenase
D. Aconitase

Q47. The rate-limiting enzymes of glycolysis are

A. Pyruvate kinase
B. Phosphofructokinase
C. Hexokinase
D. A, B, and C
E. B and C

Q48. Pyruvate kinase deficiency may lead to

A. Hemolytic anemia
B. Thalassemia major
C. Prehepatic jaundice
D. B and C

Q49. In gluconeogenesis, carboxylation of oxaloacetate by pyruvate carboxylase requires which of the following coenzymes?

A. NAD B. ATP
C. NADH D. ADP

Q50. Decrease in glutathione in red blood cells leads to

A. Iron deficiency anemia
B. Hemolytic anemia
C. Thalassemia trait
D. All of the above

Q51. Normal fasting blood glucose and glycosuria indicate

A. Diabetes mellitus
B. Renal glycosuria
C. Diabetes insipidus
D. A or C

Q52. A 54-year-old patient presented with a lack of energy, weakness, tiredness, loss of weight, and polyuria. A physician is likely to think about which of the following laboratory tests to arrive at a specific diagnosis?

A. Kidney function tests
B. Liver function tests
C. Diabetic profile tests
D. Thyroid panel tests

Q53. A 65-year-old diabetic patient under treatment, suffered from nausea, rapid pulse, sweating, weakness, shaking, and epigastric discomfort, about 3 hours following food and required intake of glucose. These symptoms probably were due to

A. Hypoglycemia B. Hyperglycemia
C. Renal glycosuria D. Both B and C

Q54. A 46-year-old woman with type II diabetes mellitus on insulin therapy, complained of repeated episodes of sleep disturbances, night sweats, mental confusion, and headache, 3–4 hours following food. What is the most likely cause of this women's symptoms

A. Hyperglycemia
B. Reactive hypoglycemia
C. Insulin resistance syndrome (syndrome X)
D. A or B

Q55. Glucose can be synthesized from all, *except*

A. Glycerol B. Amino acids
C. Lactic acid D. Acetoacetate

Q56. Insulin does not promote the transport of glucose in

A. Muscles B. Hepatocytes
C. Red blood cells D. Adipose tissue
E. All *except* C

Answers

With reasons given only of specific MCQs related to case studies

1. A **2.** C **3.** B **4.** B **5.** B **6.** D **7.** B **8.** B **9.** D **10.** B
11. A **12.** D **13.** D **14.** B **15.** C **16.** D **17.** B **18.** C **19.** B **20.** C
21. A **22.** B **23.** C **24.** D **25.** C **26.** A **27.** B **28.** C **29.** A **30.** C
31. C **32.** B **33.** D **34.** D **35.** A **36.** A **37.** B **38.** D **39.** C **40.** C
41. A **42.** A **43.** B **44.** D **45.** A **46.** C **47.** D **48.** A **49.** C **50.** B
51. B

52. C: Reason: Since the symptoms are related to “diabetes mellitus”.

53. A: Reason: The symptoms are related to hypoglycemia, which could be treated with instant intake of glucose.

54. B: Reason: Symptoms are post-prandial and are related to hypoglycemia

55. D **56.** C

Case Studies

Case 1: Answer the questions related to the following laboratory report and symptoms.

A 52-year-old obese man presented with complaints of lack of energy and loss of weight during the past two months and increased frequency of urination during night hours. He was advised to take routine diabetic tests, and the laboratory test results were as follows:

	Reference range (Normal range)
Blood glucose (fasting): 180 mg/dl	70–110 mg/dl
Blood glucose: 2 hours after lunch: (PP): 278 mg/dl	Up to 140 mg/dl
Urine sugar (fasting): Present, +	Absent
Urine sugar (PP): Present ++	Absent
Glycosylated hemoglobin: 8.5 %	4–7%

1. What is the probable diagnosis?

Ans: The patient is obese and the laboratory test results indicated that fasting blood glucose was > 126 mg/dl, and PP blood glucose was >200 mg/dl. Similarly, the presence of glucose PP urine (glycosuria) indicates the renal threshold of glucose (150–170 mg/dl) was crossed due to hyperglycemia following food intake. Hence this is a case of **type II diabetes mellitus**. (Ref: The criteria for the diagnosis of diabetes mellitus and ADA criteria).

2. What is the mechanism behind the increase in blood glucose?

Ans: The patient was obese and probably was suffering from insulin resistance. Since sufficient insulin was not available for body cells to use glucose, the level of blood glucose was increased.

3. Why glucose was present in the urine of this patient?

Ans: Renal threshold for blood glucose is 150–170 mg/dl. When blood sugar rises above the renal threshold of glucose, glucose appears in the urine. Since the blood sugar PP of this patient was 278, it was obvious that the renal threshold was crossed, hence in urine glucose was present.

4. What is the clinical significance of high glycosylated hemoglobin?

Ans: Blood values of HbA1c indicate average plasma glucose concentration of the previous 4–6 weeks. Blood HbA1c values do not change concerning recent food or glucose intake or diabetic drugs. Blood HbA1c values of more than 6.5% indicate poor glycemic control of the patient. Glycemic control means the maintenance of blood glucose values within normal range by the patient with the use of proper diet and drugs. Patients may manage to bring blood glucose levels within normal range by using the appropriate diabetic drugs, however, a physician can find out the glycemic control of the patient by blood HbA1c values.

High glycosylated hemoglobin indicated long-term hyperglycemia. He was suffering from type II diabetes mellitus and required proper glycemic control to avoid uncontrolled diabetic complications.

5. What are the additional tests required and What was the probable line of treatment?

Ans: Due to uncontrolled diabetes mellitus, the patient suffers from nephropathy, neuropathy, retinopathy, and atherosclerosis.

Hence following additional tests are recommended:

Nephropathy-related tests: Blood (serum or plasma) urea nitrogen, and serum creatinine.

Atherosclerosis-related tests: Serum total cholesterol, LDL-cholesterol, and HDL-cholesterol.

Neuropathy-related test: By consulting a neurologist

Retinopathy-related tests: By consulting an ophthalmologist.

BAQ: Show horizontal integration of symptoms and test reports of case 1 patient with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy
Possible effects of hyperglycemia on vision (loss), nerves (degeneration), blood vessels (thickening), kidneys (risk of renal disease)

Horizontal integration with physiology
Hyperglycemia, glycosuria, polyurea

Horizontal integration with nutrition
A suggestion of a diet free from high glycemic sugars like glucose, sucrose, maltose, and fructose (in table sugar, sweets, soft drinks, cakes, pastries, sweet fruits, etc.)

BAQ: Show vertical integration of symptoms and test reports of case 1 patient with pharmacology preventive medicine and pathology subjects.

Ans: Pharmacology: Study of use of appropriate drugs to treat type II diabetes mellitus.

Preventive medicine: Study of learning preventive measures like a good diet and exercises to control normal body mass index (BMI) to prevent insulin resistance (in type II diabetes mellitus).

Pathology: Study of causes of diabetes mellitus, diagnosis and prognosis.

Case 2: Answer the questions related to the following laboratory report and symptoms.

A 45-year-old obese female visited a pathology laboratory for routine blood glucose testing. The test reports were as follows:

		Reference range (Normal range)
Blood glucose (fasting):	138 mg/dl	70–100 model
Blood glucose (PP):	176 mg/dl	Up to 140 mg/dl
Urine glucose (fasting):	Absent	Absent
Urine glucose (PP):	Present, Trace	Absent

1. What is the probable diagnosis?

Ans: The laboratory test results indicated that fasting blood glucose was > 126 mg/dl, PP blood glucose was < 200 mg/dl, and in urine, glucose was not present. Since blood sugar PP was <200, these test values indicated a case of **"deranged glucose metabolism"** due to obesity. (Ref: The criteria for the diagnosis of diabetes mellitus and ADA criteria).

2. What is the biochemical basis for the mild hyperglycemia observed in this case?

Ans: The mild hyperglycemia observed in this case was due to insulin resistance caused by obesity.

3. What is the probable line of treatment?

Ans: An appropriate diet free from high glycemic sugars, and appropriate exercises (to decrease weight) are prescribed to control blood glucose levels. After 3 months, determination is of blood sugar (F) and (PP) necessary to see the effects of diet control and exercise.

Case 3: Answer the questions related to the following laboratory report and symptoms:

A 16-year-old girl was in good health, but lately, she did not feel well. She lost significant weight and complained of undue thirst and excessive urination during the night hours. On examination by a physician, it was found that she was dehydrated, her skin was cold, and her breath had a fruity odor. The following laboratory tests were performed in the morning:

	Reference range (Normal range)
Blood sugar (fasting): 593 mg/dl	70–110 mg/dl
Urine sugar (fasting): Present, ++++	Absent
Urine ketone bodies (fasting): Present, +++	Absent

1. What is the probable diagnosis?

Ans: The laboratory test results indicated that fasting blood glucose was very high and >126 mg/dl. According to the age of the patient and no history of obesity indicates this is a case of **type II diabetes mellitus**. (Ref: The criteria for the diagnosis of diabetes mellitus and ADA criteria).

2. What is the biochemical basis behind the increase in blood glucose?

Ans: Failure of beta cells of the pancreas to secrete normal amounts of insulin or presence of specific antibodies of insulin that prevented insulin action of introducing glucose molecules to cells for energy production.

3. Why glucose was present in the urine of this patient?

Ans: Normal renal threshold for blood glucose is 150–170 mg/dl. Since fasting blood sugar was 593, it was obvious that the renal threshold was crossed, hence in urine, glucose was present.

4. Why ketone bodies were present in the fasting urine?

Ans: In the case of a normal individual detectable amount of glucose is not present in urine for 24 hours. Since glucose was not used by the body cells; in the absence of insulin, free fatty acids (FFA) were used excessively for the generation of ATP molecules as an energy source. In the metabolism of FFA, acetyl-CoA forms, which is partly converted to ketone bodies, which are excreted in the urine.

5. What are the additional tests required and what is the probable line of treatment?

Ans: The following additional tests are required to finsd out if this patient is suffering from acidosis: Blood pH, serum bicarbonate, and arterial blood levels of carbon dioxide and oxygen. The patient required appropriate insulin therapy.

Case 4: Answer the questions related to the following laboratory report and symptoms:

A 64-year-old diabetic man was on hypoglycemic therapy. One day, two hours after lunch, he suffered from specific symptoms such as nausea, lightheadedness, rapid pulse, sweating, weakness, and confusion. His blood sample was tested for random blood glucose. The laboratory test report was as follows:

	Reference range (Normal range)
Blood glucose (random): 45 mg/dl.	70–150 mg/dl

1. What is the probable diagnosis?

Ans: The random blood glucose was significantly less than 70 mg/dl and indicated a case of **hypoglycemia**.

2. What is the biochemical basis behind a significant decrease in blood glucose?

Ans: The diabetic patient was on hypoglycemic therapy. The food intake and gastrointestinal health of the patient must be normal, otherwise, the usual dose of the prescribed drug will decrease blood sugar significantly.

3. What is the probable first line of treatment?

Ans: About 10–15 g of glucose dissolved in drinking water should be given to the patient and time is noted. Blood glucose should be tested after 15 minutes to make sure that level of random blood glucose is normal (>70 mg/dl).

Chapter

5

Lipids

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

Competency achievement: The student should be able to:

BI4.1: Describe and discuss the main classes of lipids: Relevant to the human systems and their functions: Essential and non-essential fatty acids, cholesterol, hormonal steroids, triglycerides, major phospholipids, sphingolipids

INTRODUCTION

Lipids are a group of molecules and compounds that include fats, oils, monoglycerides, diglycerides phospholipids, cholesterol, waxes, sterols, fat-soluble vitamins, etc. The functions of lipids include acting as the storage form of energy, structural components of cell membranes, and basic components for the formation of steroid hormones and bile salts.

CLASSIFICATION AND IMPORTANCE

LAQ: What are lipids? Classify lipids with examples and physiological importance.

Ans: The lipids are a group of organic substances of fatty nature that are (1) Insoluble in water, (2) soluble in fat solvents, such as ether, alcohol, chloroform, and benzene, (3) related to the fatty acids (either actually or potentially) as esters and (4) utilizable in metabolism by living organisms.

The lipids can be classified into three main groups: (1) Simple lipids, (2) Compound or conjugate lipids, and (3) Derived lipids. These main groups can be divided as follows:

Simple Lipids

These are esters of fatty acids with certain alcohols. Simple lipids are further classified according to the nature of alcohol, as follows:

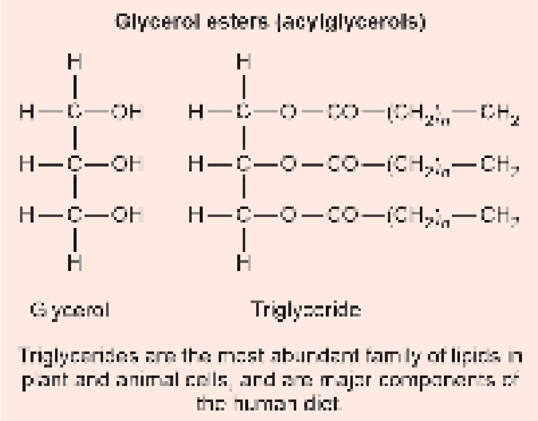
Fats and Oils (Fig. 5.1)

These are esters of fatty acids and glycerol. If the fat is liquid at ordinary room temperature, it is called an oil. Most of the triglycerides which occur in nature are mixed triglycerides; that is, they contain two or three different fatty acids in the molecule.

Examples: Tripalmitin, tristearin, animal fats, coconut oil, butter fat, etc.

Waxes: These are esters of fatty acids with long-chain aliphatic alcohol or cyclic alcohol.

Examples: True waxes, cholesterol esters, vitamin D esters, vitamin A, and its carotenol esters.


Glycerol esters (acylglycerols)
Glycerol
Triglyceride

Triglycerides are the most abundant family of lipids in plant and animal cells, and are major components of the human diet

Fig. 5.1: Glycerol and triglyceride

COMPOUND (CONJUGATE) LIPIDS

These lipids on hydrolysis yield other substances such as phosphoric acid, nitrogenous bases, galactose, sulfuric acid, etc. in addition to fatty acid and alcohol.

Examples: Phospholipids, cerebrosides, sulpholipids, etc.

Phospholipids (Fig. 5.2)

1. *Lecithins:* These contain glycerol and fatty acids as do the simple fats, but they also contain phosphoric acid and choline (nitrogenous component). These are widely distributed in the cells of the body but are particularly important in fat metabolism by the liver.
2. *Cephalins:* Cephalins differ from lecithins only in that ethanolamine replaces choline. Cephalins are components of certain lipoproteins. The lipoprotein thromboplastin, which is an important factor in blood coagulation, contains a relatively high level of cephalin.
3. *Phosphatidylserine:* A cephalin-like phospholipid that contains the amino acid serine rather than ethanolamine. It has been found in brain tissue.
4. Phosphatidylinositol (inositol): Inositol is an important component of lipositol, and these occur in the brain tissue.

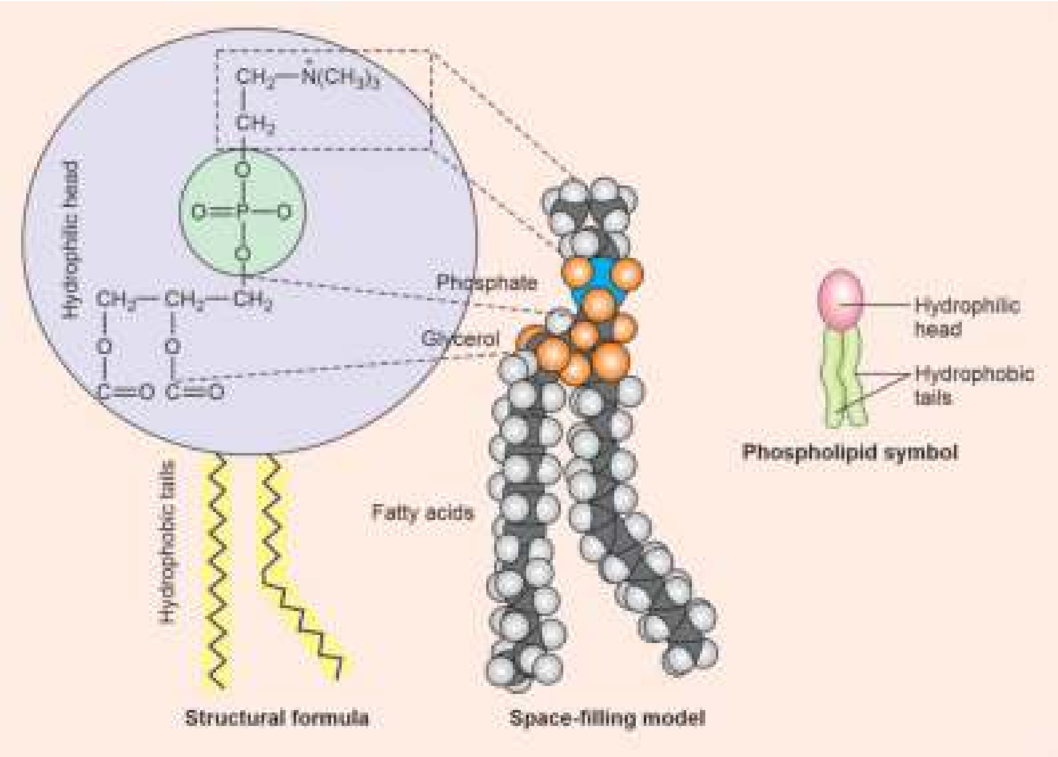

Hydrophilic head
Phosphate
Glycerol
Hydrophobic tails
Fatty acids
Structural formula
Space-filling model
Hydrophilic head
Hydrophobic tails
Phospholipid symbol

Fig. 5.2: Phospholipid

5. Cardiolipin: This is an important phospholipid of the mitochondrial membrane. It is phosphatidyl glycerol in which two phosphatidic acids are joined by a molecule of glycerol.
6. Plasmalogens: These compounds possess fatty aldehyde in place of fatty acid at the alpha-position. These compounds constitute as much as 10% of the phospholipid of the brain and muscle.

DERIVED LIPIDS

Derived are formed in the hydrolysis of simple or compound lipids. The derived lipids also include substances associated with lipids in nature, such as carotenes, vitamins A, D, E, and K.

Examples of derived lipids: Saturated and unsaturated fatty acids, bile acids, glycerol, oil-soluble vitamins, and steroids

Some important saturated fatty acids in this series are as follows:

Table 5.1: Chemical formula of saturated fatty acids

Saturated fatty acid	Chemical formula
1. Acetic	CH_3COOH
2. Butyric	C_3H_7COOH
3. Caproic	$C_5H_{11}COOH$
4. Lauric	$C_{11}H_{23}COOH$
5. Palmitic	$C_{15}H_{31}COOH$
6. Stearic	$C_{17}H_{35}COOH$

Unsaturated Fatty Acids

Unsaturated fatty acids may be further subdivided under the number of double bonds.

1. Monounsaturated fatty acids: Oleic series: One double bond
   General formula: C_nH_{2n-1} COOH
   Example: Oleic acid: $CH_3(CH_2)_7CH = CH(CH_2)_7COOH$
2. Polyunsaturated fatty acids:
   - Linoleic series: Two double bonds
     General formula: $C_nH_{2n-3}COOH$
     Example: Linoleic acid:
     $CH_3(CH_2)_4CH = CHCH_2CH = (CH_2)_7–COOH$
   - Linolenic series: Three double bonds
     General formula: $C_nH_{2n-5}COOH$
     Example: Linolenic acid:
     $CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_7COOH$
   - Fatty acids with four double bonds
     General formula: $C_nH_{2n-7}COOH$
     Example: Arachidonic acid:
     $CH_3(CH_2)_4(CH = CHCH_2)_4(CH_2)2COOH$
3. Eicosanoids: Derived from eicosa (20-carbon) polyenoic fatty acids

The following are important biological functions of lipids:

1. They serve as the reservoir of high energy value. Its calorific value is 9-kilo calories/g as compared to carbohydrates which have a calorific value of 4 kcal.
2. They can be stored in concentrated form in water free state (as compared to carbohydrates) in the adipose tissue.
3. They are important components of cell membranes.
4. They form an important constituent of nervous tissue.
5. They form an insulating and protective coating in the subcutaneous tissues and around certain organs (e.g. kidneys).
6. In the form of oil-soluble vitamins (A, D, E, K) and essential fatty acids (linoleic and linolenic acid), they are important dietary constituents.
7. Lipoproteins (a combination of lipids and proteins) are important constituents of cell membranes and mitochondria.

BAQ: What are simple lipids? Give examples. Enumerate their functions.

Ans: These are esters of fatty acids with certain alcohols. Simple lipids are further classified according to the nature of alcohol, as follows: 1. Fats (triglycerides) and 2. Waxes (Fig. 5.1).

Fats (Fig. 5.1)

These are esters of fatty acids and glycerol. If the fat is liquid at ordinary room temperature, it is called an oil. Most of the triglycerides which occur in nature are mixed triglycerides; that is, they contain two or three different fatty acids in the molecule.

Examples: Tripalmitin, tristearin, animal fats, coconut oil, butter fat, etc.

Since all of the true fats contain glycerol, their chemical and physical properties are determined by the nature of fatty acid components. Triglycerides have a specific gravity of less than one (about 0.86). They are, therefore, lighter than water. The melting point of fats is dependent on the chain length of the component fatty acids and their degree of unsaturation.

Functions of triglycerides

1. Triglycerides are stored in the adipose tissues and act as a very important source of energy.
2. In general blood circulation, triglycerides release glycerol and free fatty acids, which can be used by the body cells for the production of ATP molecules.

Waxes

These are esters of fatty acids with long-chain aliphatic alcohol or cyclic alcohol.

Examples of waxes: True waxes, cholesterol esters, vitamin D esters, and vitamin A and its carotenol esters.

Functions of waxes

1. Waxes play an important role as an insulating material and repel water molecules and microorganisms.
2. Transport cholesterol to various parts of the body in the form of cholesterol esters.
3. Play an important role in the metabolism of oil-soluble vitamins as respective esters.

LAQ: What are compound lipids? Give examples. Enumerate their functions.

Ans: Compound lipids on hydrolysis yield other substances such as phosphoric acid, nitrogenous bases, galactose, sulfuric acid, etc., in addition to fatty acid and alcohol.

Examples of compound lipids: Phospholipids, sphingomyelins, cerebrosides, sulfolipids, lipoproteins, etc.

Following are examples of phospholipids and their respective functions:

1. Lecithins: These contain glycerol and fatty acids as do the simple fats, but they also contain phosphoric acid and choline (nitrogenous component). These are widely distributed in the cells of the body but are particularly important in fat metabolism by the liver.
2. Cephalins: Cephalins differ from lecithins only in that ethanolamine replaces choline. Cephalins are components of certain lipoproteins. The lipoprotein thromboplastin, which is an important factor in blood coagulation, contains a relatively high level of cephalin.
3. Phosphatidylserine: A cephalin-like phospholipid that contains the amino acid serine rather than ethanolamine. It has been found in brain tissue.
4. Phosphatidylinositol (lipositol): Inositol is an important component of lipositol, and these occur in the brain tissue.
5. Cardiolipin: This is an important phospholipid of the mitochondrial membrane. It is phosphatidyl glycerol in which two phosphatidic acids are joined by a molecule of glycerol.
6. Plasmalogens: These compounds possess fatty aldehyde in place of fatty acid at the alpha position. These compounds constitute as much as 10% of the phospholipid of the brain and muscle.

NOTE

Due to their amphipathic nature, phospholipids can react with both polar and nonpolar compounds in the cell. They are an important component of the cell membrane, bile and participate in the absorption of lipids in the intestine and also participate in the synthesis of lipoproteins in the liver. They play an important role in reverse cholesterol transportation. Lecithin participates in the blood clotting mechanism and arachidonic acid is a precursor for the synthesis of eicosanoids.

Sphingomyelins

These are found in large quantities in brain and nerve tissues. No glycerol is present. Sphingomyelins do not contain glycerol. On hydrolysis, the sphingomyelin yields a fatty acid, phosphoric acid, choline, and a complex amino alcohol called sphingosine (or sphingol).

Functions of sphingolipids (or sphingol)

Sphingomyelins are the main sphingolipids of human cells. These lipids are enriched in the plasma membrane and in endosomes, where they perform following various functions.

1. Sphingolipids protect the cell surface against harmful environmental factors. They form a mechanically stable and chemically resistant outer layer of the plasma membrane lipid bilayer.
2. Certain complex glycosphingolipids are also involved in specific functions, such as cell recognition and signaling. The cell recognition feature depends mainly on the physical properties of the sphingolipids. Signaling involves specific interactions of the glycan structures of glycosphingolipids with similar lipids present on neighboring proteins or cells.
3. Simple sphingolipid metabolites, such as ceramide and sphingosine-1-phosphate, are important mediators in the signaling cascades involved in apoptosis, proliferation, and stress responses.
4. Sphingolipids constitute a 20–35 molar fraction of plasma membrane.

Cerebrosides (glycolipids)

These contain galactose, sphingol and a high molecular weight fatty acid. Individual cerebrosides are differentiated by the kind of fatty acid in the molecule. The four cerebrosides have been isolated. These are kerasin, cerebron, nervon, and oxynervon.

Functions of cerebrosides

1. The cerebrosides are present in the brain. These are present in higher concentrations in medullated than in nonmedullated nerve fibers.
2. The carbohydrate component of the cerebroside remains present on the outside of the cell membrane. It plays a part in cell recognition and cell-to-cell interactions.

Sulfolipids

These are similar to cerebrosides except that sulfuric acid is present as cerebronic acid ester. Sulfolipids contain sphingosine, galactose, cerebronic acid, sulfuric acid, and potassium.

Functions of Sulfolipids

Sulfolipids are involved in sodium transport, activation of the oxygen radical generating system, blood coagulation factor XII, and spermatogenesis, etc.

Lipoproteins

Lipids such as triglycerides, phospholipids, and cholesterol are water insoluble and are transported in the body in blood in combination with various specific proteins (apoproteins). These occur in the following five major forms:

1. Chylomicrons
2. Very low-density lipoproteins
3. Low-density lipoproteins
4. High-density lipoproteins

Functions of Lipoproteins

Plasma lipoproteins are in a dynamic state. They are continuously being synthesized and degraded with a rapid exchange of proteins and lipids. The functions of lipoproteins are as follows:

1. Chylomicrons: Main vehicle of absorbed lipids in the gastrointestinal tract. Chylomicrons are degraded continuously by lipoprotein lipase enzymes in blood vessels to release free fatty acids, which are used by the cells for energy purposes.

2. Very low-density lipoproteins: Main vehicle of circulation of triglycerides in the body.
3. Low-density lipoproteins: Main vehicle of circulation of cholesterol in the body.
4. High-density lipoproteins: Play a very important role in the removal of excess cholesterol and transporting it to the liver for the synthesis of bile acids.

BAQ: What are derived lipids? Give examples. Enumerate their functions.

Ans: Derived lipids: These are formed in the hydrolysis of simple or compound lipids. The derived lipids also include substances associated with lipids in nature, such as carotenes, vitamins A, D, E, and K.

Examples: Saturated and unsaturated fatty acids, bile acids, glycerol, oil soluble vitamins, and steroids.

Saturated fatty acids: *Examples:* Butyric acid, lauric acid, palmitic acid, stearic acid, etc.

Unsaturated fatty acids: The following are various types of unsaturated fatty acids.

1. Monounsaturated fatty acids: Oleic series: One double bond.
   Example: Oleic acid
2. Polyunsaturated fatty acids:
   Linoleic series: Two double bonds. *Example:* Linoleic acid.
   Linolenic series: Three double bonds. *Example:* Linolenic acid.
   - Fatty acids with four double bonds. *Example:* Arachidonic acid.
   - Eicosanoids: Derived from eicosa (20-carbon) polyenoic fatty acids.

The following are the important biological functions of lipids:

Steroids

The steroids are often found in association with fat. They are present in "unsaponifiable residue" and hence, may be separated from the fat after the fat is saponified. All the steroids have a similar cyclic nucleus resembling phenanthrene (rings I, II, and III) to which a cyclopentane ring (IV) is attached. The parent substance is designed as cyclopentanoperhydrophenanthrene (Fig. 5.3). The positions on the steroid nucleus are numbered as follows (Fig. 5.4):

1. Methyl groups are frequently attached at positions 10 and 13.
2. A side chain at position 17 is usual (as shown below in the case of cholesterol).
3. If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a sterol.
4. If it has one or more carbonyl or carboxyl groups, it is a steroid.

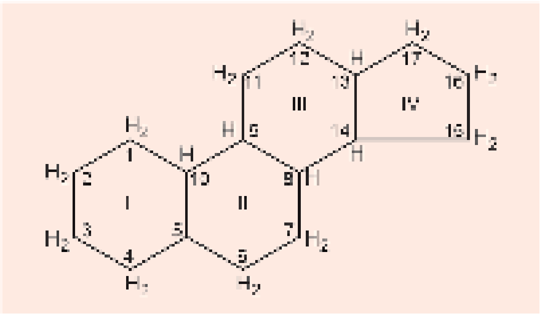


Fig. 5.3: Cyclopentanoperhydrophenanthrene

Some of the biologically important steroids are: (a) Bile acids, (b) Corticosteroids, (c) Female hormones, and (d) Male sex hormones

Cholesterol (Fig. 5.4)

Cholesterol is found exclusively in animals and humans, and it is also the main sterol. Virtually all cells and body fluids contain some cholesterol. Like other sterols, cholesterol is solid alcohol of high molecular weight and possesses the tetracycline, perhydrocyclopentanophenanthrene skeleton (Fig. 5.3). Cholesterol is the initial starting point in many metabolic pathways. These include vitamin D synthesis, steroid hormone synthesis, and bile acid metabolism.

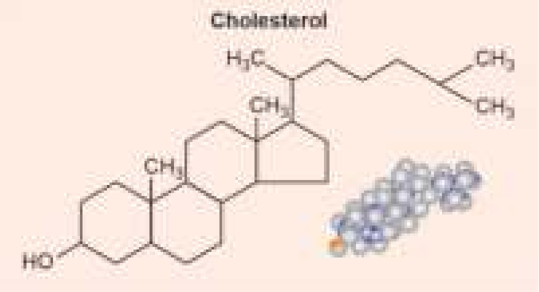


Fig. 5.4: Structural formula of cholesterol

Glycolipids

These lipids are attached to a carbohydrate molecule. Their role is to serve as markers for cellular recognition and also to provide energy. They occur when a carbohydrate chain is associated with phospholipids on the exoplasmic surface of the cell membrane. The glycolipids are found on the outer surface of all eukaryotic cell membranes.

Eicosanoids

Eicosanoids are comprised of prostanoids, leukotrienes, and lipoxins (LXs). Eicosanoids are derived from either omega-3 or omega-6 essential fatty acids. The omega-6 eicosanoids are generally pro-inflammatory. The amounts and balance of these fats in a person's diet affect the body's eicosanoid-controlled functions, with effects on cardiovascular disease, triglycerides, blood pressure, and arthritis. Anti-inflammatory drugs such as aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen act by downregulating eicosanoid synthesis.

SAQ: What is a trans configuration of fatty acids?

Ans: A trans configuration means that the next two hydrogen atoms are bound to opposite sides of the double bond. As a result, they do not cause the chain to bend much, and their shape is similar to straight saturated fatty acids (Fig. 5.5).

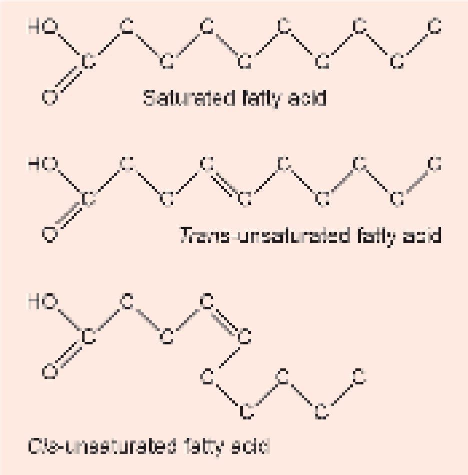


Fig. 5.5: Chains of saturated, *trans*-unsaturated, and *cis*-unsaturated fatty acids

SAQ: What are short-chain and medium-chain fatty acids?

Ans: Short-chain fatty acids (SCFA) are fatty acids with aliphatic tails of fewer than six carbons.

Medium-chain fatty acids (MCFA) are fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain triglycerides.

Q: What are long-chain and very long-chain fatty acids?

Ans: Long-chain fatty acids (LCFA) are fatty acids with aliphatic tails longer than 12 carbons.

Very-long-chain fatty acids (VLCFA) are fatty acids with aliphatic tails longer than 22 carbons.

SAQ: What are short-chain essential fatty acids (EFAs)?

Ans: Short-chain essential fatty acids (EFAs) are 18 carbons long; long-chain EFA has 20 or more carbons.

SAQ: Write a short note on trans fatty acids.

Ans: Trans fatty acids (TFAs) are unsaturated fatty acids. These are not synthesized by mammals. These TFAs are characteristically produced during the industrial hydrogenation of plant oils. Since they are also produced in bacterial metabolism, ruminant fats (e.g.

in milk) also contain about 4% trans fatty acids. It has been found that trans fats, just like saturated fats, raise the LDL cholesterol and lower the HDL cholesterol. They have also been shown to increase triglycerides. Trans fatty acids are also responsible to cause more inflammation, which is thought to occur through damage to the cell lining of blood vessels.

BAQ: Write a short note on essential fatty acids.

Ans: Fatty acids such as linoleic acid, linolenic acid, and arachidonic acid cannot be synthesized in the body from other substrates and must be supplied in food. Hence, they are called essential fatty acids. Mammals cannot introduce double bonds in fatty acids beyond carbons 9 and 10. About twenty different fatty acids are synthesized from the two essential fatty acids, linoleic acid, and linolenic acid. Omega-6 fatty acids are synthesized from linoleic fatty acid and omega 3-fatty acid is synthesized from linolenic fatty acids.

A healthy diet should contain a balance of omega-3 and omega-6 fatty acids, which helps stimulate skin and hair growth, good bone health, regulation of metabolism, and in the maintenance of the reproductive system. A typical American diet tends to contain 14–25 times more omega-6 fatty acids than omega-3 fatty acids since lots of meat is the main constituent of their food, which contains a high concentration of omega-6- fatty acid. However, the Mediterranean (Europe, Asia, and Africa regions) diet, on the other hand, has a healthier balance between omega-3 and omega-6 fatty acids, which contains whole grains, fresh fruits, vegetables, fish, olive oil, garlic, etc. Many studies have shown that people who follow a Mediterranean diet are less likely to develop heart disease. Omega-3 fatty acids help to reduce inflammation, and some omega-6 fatty acids tend to promote inflammation.

BAQ: What are essential fatty acids? Write examples of essential fatty acids. Why humans are not able to synthesize essential fatty acids?

Ans: Essential fatty acids cannot be synthesized by human cells. However, these are essential for normal metabolic activities and growth of the body. Essential fatty acids are long-chain fatty acids containing twenty and more than twenty carbon atoms with double bonds. Examples of essential fatty acids are linoleic acid, linolenic acid, and arachidonic acid.

Humans can easily make saturated fatty acids or monounsaturated fatty acids with a double bond at the omega-9 position but do not have the enzymes necessary to introduce a double bond at the omega-3 position or omega-6 position. Essential fatty acids are polyunsaturated fatty acids and are the parent compounds of the omega-6 and omega-3 fatty acid series, respectively.

BAQ: Describe the importance of essential fatty acids. What are the symptoms of essential fatty acid deficiency?

Ans: The essential fatty acids are used to make compounds such as prostaglandins. The brain has an increased amount of linoleic and alpha-linolenic acid derivatives. Changes in the levels and balance of these fatty acids due to a typical diet rich in omega-6 and poor in omega-3 fatty acids is alleged to be associated with depression and behavioral changes

Symptoms of essential fatty acid deficiency in humans include impairment of lipid transport and the development of skin lesions. Abnormal metabolism related to dietary insufficiency is also observed in hepatorenal syndrome, cystic fibrosis, Crohn’s disease, cirrhosis of the liver, and multisystem neuronal degeneration.

LAQ: What are steroids and sterols? Give examples with appropriate structural diagrams.

Ans: The steroids are often found in association with fat. They are present in “unsaponifiable residue” and hence, may be separated from the fat after the fat is saponified. All the steroids have a similar cyclic nucleus resembling phenanthrene (rings I, II, and III) to which a cyclopentane ring (IV) is attached. The parent substance is designed as cyclopentanoperhydrophenanthrene (Fig. 5.3). The positions on the steroid nucleus are numbered as follows:

1. Methyl groups are frequently attached at positions 10 and 13 (Figs 5.4 and 5.6).
2. A-side chain at position 17 is usual (as shown below in the case of cholesterol).
3. If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a sterol.
4. If it has one or more carbonyl or carboxyl groups, it is a steroid.

Some of the biologically important steroids are: (a) Bile acids, (b) corticosteroids, (c) female hormones, and (d) male sex hormones

BAQ: Write a short note on cholesterol.

Ans: Cholesterol is found exclusively in animals and humans, and it is also the main sterol. Virtually all cells and body fluids contain some cholesterol. Like other sterols, cholesterol is solid alcohol of high molecular weight and possesses the tetracycline, perhydrocyclopentanophenanthrene

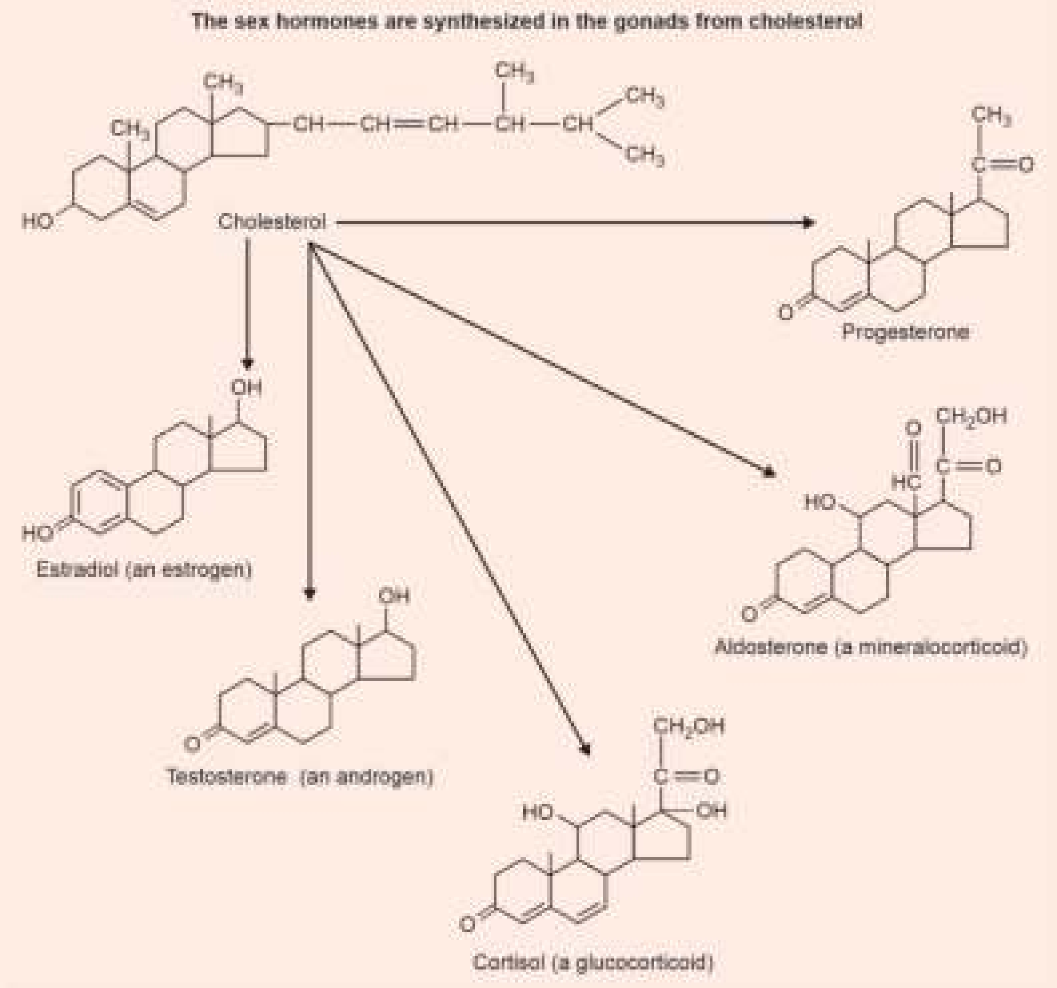

The sex hormones are synthesized in the gonads from cholesterol
CH_3
CH_3
CH_3
CH_3
CH—CH═CH—CH—CH
CH_3
HO
Cholesterol
CH_3
C═O
O
Progesterone
OH
HO
Estradiol (an estrogen)
O
CH_2OH
HC
C═O
HO
O
Aldosterone (a mineralocorticoid)
OH
O
Testosterone (an androgen)
CH_2OH
C═O
HO
OH
O
Cortisol (a glucocorticoid)

Fig. 5.6: Various steroids formed from cholesterol

skeleton (Fig. 5.3). Cholesterol is the initial starting point in many metabolic pathways. These include vitamin D synthesis, steroid hormone synthesis, and bile acid metabolism (Fig. 5.6).

Dietary intake and biosynthesis of cholesterol mainly by the hepatic cells are responsible for the levels of cholesterol in the body. Biosynthesis of cholesterol generally takes place from acetyl-CoA molecules in the endoplasmic reticulum of hepatic cells.

Animal products, especially meat, egg yolk, seafood, and whole-fat dairy products, provide the bulk of dietary cholesterol. Esterified cholesterol in the diet is rapidly hydrolyzed in the intestine to free cholesterol and free fatty acids (FFA) by cholesterol esterases in pancreatic and small-intestinal secretions. Free cholesterol and also the esterified form of cholesterol are absorbed in the small intestine and enter general blood circulation through lacteals as a component of chylomicrons. Cholesterol is added to the general blood circulation also by hepatic cells in the form of lipoproteins such as very low-density cholesterol (VLDL), low-density cholesterol (LDL), and high-density cholesterol (HDL). HDL cholesterol is also added by the small intestine.

Increased LDL-cholesterol is captured by macrophages in the blood vessels, leading to the deposition of LDL-cholesterol in the walls of arteries. Thus, an increase in total blood cholesterol leads to atherosclerosis, which means the narrowing of blood vessels due to cholesterol deposits. Atherosclerosis may lead to heart disease.

SAQ: What are glycolipids?

Ans: Glycolipids are a combination of lipids and carbohydrates. Their role is to serve as markers for cellular recognition and also to provide energy. They occur when a carbohydrate chain is associated with phospholipids on the exoplasmic surface of the cell membrane. The glycolipids are found on the outer surface of all eukaryotic cell membranes. The carbohydrate structure of the glycolipid is controlled by the glycosyltransferases that add the lipids. The enzyme glycosylhydrolases then modify the formed molecule. Glycolipids extend from the phospholipid bilayer into the aqueous environment outside the cell, where they act as a recognition site for specific chemicals as well as help to maintain the stability of the membrane and also help to attach cells to form tissues.

Competency achievement: The student should be able to:

BI4.6: Describe the therapeutic uses of prostaglandins and inhibitors of eicosanoid synthesis

BAQ: What are eicosanoids?

Ans: Eicosanoids are derived from either omega-3 or omega-6 essential fatty acids. These are synthesized by body cells in response to inflammation, allergic conditions, fever, etc. Eicosanoids are comprised of prostanoids, leukotrienes, and lipoxins (LXs). The amounts and balance of these fats in a person's diet affect the body's eicosanoid-controlled functions, with effects on cardiovascular disease, triglycerides, blood pressure, and arthritis. Anti-inflammatory drugs such as aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen act by downregulating eicosanoid synthesis. Eicosanoids are not stored within cells but are synthesized as required. Following two families of enzymes catalyze fatty acid oxygenation to produce the eicosanoids:

1. Cyclooxygenase (COX), which generates the prostanoids and
2. Lipoxygenase (LOX) generates the leukotrienes.

SAQ: What are prostanoids?

Ans: The prostanoids are part of a family of biologically active lipids derived from the action of cyclo-oxygenases or prostaglandin synthases upon the twenty-carbon essential fatty acids or eicosanoids. They can be further subdivided into three main groups, prostaglandins, prostacyclins, and thromboxanes. Each of these is involved in some aspect of the inflammatory response. In general, prostaglandins occur at very low levels in tissues, of the order of nanomolar concentrations, but they have profound biological activities.

BAQ: What are prostaglandins? What are the functions of prostaglandins?

Ans: Prostaglandins are derived from either omega-3 or omega-6 essential fatty acids. These are synthesized by body cells in response to inflammation, allergic conditions, fever, etc.

Each prostaglandin is named using the prefix 'PG' followed by a letter A to K depending on the nature and position of the substituents on the ring. Thus PGA to PGE and PGJ have a keto group in various positions on the ring. These are further distinguished by the presence or absence of double bonds or hydroxyl groups in various positions in the ring. PGF has two hydroxyl groups, while PGK has two keto substituents on the ring. PGG and PGH are bicyclic endoperoxides, which are heterocycle structures containing a peroxide -O-O- residue in the ring An oxygen bridge between carbons 6 and 9 distinguishes prostacyclin (PGI).

Cyclo-oxygenase (COX) enzyme catalyzes the conversion of the free essential fatty acids to prostaglandins. Several drugs, such as aspirin and NSAID lower inflammation by blocking prostaglandin synthesis.

Functions of Prostaglandins

Prostaglandins act as vasodilators and inhibit the aggregation of blood platelets. These are involved in the inflammation processes, following microbial infections. Inflammation is the response of the immune system to infection and injury and has been observed in the pathogenesis of various clinical conditions such as stroke, cancer, arthritis, cardiovascular and neurodegenerative diseases. Inflammation leads to the removal of harmful factors and the restoration of normal physiological functions and tissue structures. Prostaglandins also prevent the formation of micro-clots in the blood vessels. These are also involved in the regulation of the contraction of smooth muscle tissue.

SAQ: Enumerate pharmaceutical uses of prostaglandins.

Ans: The various pharmaceutical uses of prostaglandins are to:

1. Reduce inflammation
2. Decrease blood pressure
3. Inhibit platelet aggregation
4. Promote healing of wounds and infections
5. Control allergic reactions
6. Control auto-immune disorders

SAQ: Why cyclo-oxygenase is known as a "suicide enzyme"?

Ans: Cyclo-oxygenase is known as a "suicide enzyme" since it undergoes self-catalyzed destruction (irreversible inhibition by the end product of the catalytic reaction) leading to the "switching off" of prostaglandin activity. The inactivation of prostaglandins is done by 15-hydroxyprostaglandin dehydrogenase. Non-steroid anti-inflammatory drug (NSAID) such as aspirin blocks the action of cyclo-oxygenase, leading to a decrease in the synthesis of prostanoids and control of increased temperature and inflammation levels in the body of a patient.

SAQ: Enumerate names of inhibition of prostaglandin synthesis.

Ans: Following non-steroidal anti-inflammatory drugs (NSAIDs): Aspirin, ibuprofen, naproxen, fenoprofen, indomethacin.

LAQ: Describe various properties of lipids used in clinical laboratory experiments.

Ans: The following are the important chemical properties of lipids on which the laboratory determinations are based:

1. **Enzymatic hydrolysis:** Triglycerides are split into glycerol and fatty acids by the lipases at alkaline pH (7.5–8.5) and in the presence of water. The hydrolysis takes place in a stepwise manner. Diglycerides are formed first, followed by mono-glycerides, and finally, the mono-glycerides are split to free glycerol and fatty acids. Since the concentration of glycerol formed is directly proportional to triglyceride concentration, the laboratory methods are based on the determination of glycerol.
2. **Saponification:** The hydrolysis of triglycerides by alcoholic potassium hydroxide (or by sodium hydroxide) is called saponification, and the end products are glycerol and potassium (or sodium) salts called soaps. This property of triglyceride can also be used for its quantitative determination by determining glycerol (which is directly proportional to the concentration of triglycerides in the specimen).
3. **Adsorption:** Phospholipids are specifically adsorbed on alumina. This property is used in the determination of serum triglycerides (by the methods based on glycerol determination) to remove phospholipids since phospholipids also contain glycerol.
4. **Liebermann-Burchard reaction:** Cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and concentrated sulfuric acid to form a green-colored compound. This principle is used in the colorimetric determination of serum total cholesterol.
5. **Precipitation of certain lipoproteins:** The combination of phosphotungstic acid and magnesium chloride or heparin and manganese chloride is used to precipitate chylomicrons, LDL, and VLDL in serum. The supernatant contains HDL. This principle is used to determine HDL cholesterol.
6. **Extraction of lipids:** Ether, alcohol, and isopropanol are used to extract lipids in serum. During the extraction of lipids, proteins are also precipitated by these organic solvents so that the protein-free filtrate contains triglycerides, phospholipids, total cholesterol, and free fatty acids.
7. **Electrophoretic fractionation of lipoproteins:** Serum lipoproteins, when subjected to electrophoresis on agarose gel (or cellulose acetate paper), separate into the following fractions (Fig. 5.7):

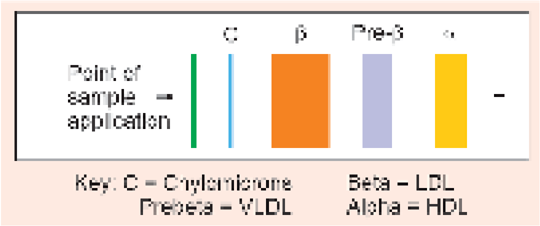


Fig. 5.7: Electrophoretic fractionation of lipoproteins

Competency achievement: The student should be able to:

BI4.2: Describe the processes involved in the digestion and absorption of dietary lipids and also the key features of their metabolism

DIGESTION AND ABSORPTION OF LIPIDS

LAQ: Write a note on the digestion of lipids.

Ans: The normal average intake of lipids (of an adult) is about 60–70 g per day. Of this, about 90% is fat, and the rest of the dietary lipid is composed of phospholipids, free fatty acids, cholesteryl esters, and free cholesterol.

Digestion of Lipids in Stomach

The digestion of lipids is initiated in the stomach by lingual lipase (which originates from the serous glands at the back of the tongue) and gastric lipase, secreted by a chief cell of the gastric mucosa. These lipases act on fat-containing short-chain fatty acids at neutral pH, to form mainly free fatty acids. Although the low pH in the stomach is unfavorable for the action of gastric lipase, after feeding (particularly ingestion of milk) these lipases become active (at pH 3.0 to 6.0). Milk fat (containing short and medium-chain fatty acids) is particularly a good substrate for gastric lipase. The released hydrophilic short- and medium-chain fatty acids are absorbed through the stomach wall and enter the portal vein. Longer-chain fatty acids dissolve in the fat droplets and pass on to the duodenum.

NOTE

Gastric lipase is secreted by the chief cells of the stomach. It is an acidic lipase at pH 3–6. Gastric lipase and lingual lipase both are two acidic lipases. These lipases do not require bile acid or colipase for optimum enzymatic activity. However, pancreatic lipase is an alkaline lipase and requires bile acids and colipase for the digestion of lipids in the small intestine.

Digestion of Lipids in the Intestine

The stomach contents (chyme) are intermittently introduced during digestion into the duodenum through the pyloric valve. The acidic nature of chyme is changed to alkaline due to the biliary and pancreatic secretions. This shift of pH is necessary for the activity of the enzymes contained in pancreatic and intestinal juice.

During digestion, the gall bladder contracts and supplies bile to the duodenum by way of the common bile duct. Bile acids in bile emulsify fats in the intestine and dissolve fatty acids. Lipase in pancreatic secretions acts effectively on the emulsified lipids. Pancreatic lipase, with the help of colipase (secreted by the pancreas), acts on triglycerides to form 2-monoacylglycerol and free fatty acids. Lipid esterase present in pancreatic juice acts on monoacylglycerols, cholesteryl esters, and oil-soluble vitamin esters to liberate free fatty acids (in the presence of bile salts). Phospholipases in pancreatic juice act on phospholipids to form free fatty acids and lysophospholipids. Lysophospholipases act on lysophospholipids to liberate free fatty acids and glycerophosphoric choline. Pancreatic cholesterol esterase cleaves cholesteryl esters to produce free fatty acids and cholesterol.

LAQ: Write a note on the absorption of lipids in the small intestine.

Ans: Absorption of lipids: The end products of lipid digestion, the 2-monoacylglycerols, fatty acids, and small amounts of 1-monoacylglycerols leave the oil phase of the lipid emulsion and diffuse into the mixed micelles and liposomes consisting of bile salts, phosphatidylcholine, and cholesterol. The micelles are soluble and are ultimately absorbed into intestinal epithelium. Bile acids are essential for the absorption of digested lipids. The bile acids pass on to the ileum, where most are absorbed into the enterohepatic circulation by an active transport system.

Resynthesis of lipids in the intestinal mucosal cells: The short and medium-chain fatty acids (<10 carbons) after absorption, enter the portal circulation and are transported to the liver in a bound form to albumin. The long-chain fatty acids are activated by thiokinase and combine with 2-monoacylglycerols to produce triacylglycerols. These reactions are catalyzed by acyltransferases. Similarly, free cholesterol reacts with free fatty acids to form esterified cholesterol, and phospholipids are generated from the absorbed lysophospholipids.

The resynthesized lipids are hydrophobic. They form lipid droplets surrounded by a thin layer of apoproteins A-1 and B-48 and phospholipids. These lipid droplets, known as chylomicrons, migrate to the plasma membrane of intestinal mucosal cells. They are released into the lymphatic vessels by exocytosis. Chylomicrons enter the large veins through the thoracic duct. Through blood circulation, they flow to the lungs, peripheral tissues (muscle, adipose tissue), and, finally to the liver.

The chylomicrons are finally deposited either in the liver or in the fat storage depots (adipose tissue), which in health, form about 15% of the body weight. Lipoprotein lipase in the adipose tissue is responsible for the clearance of chylomicrons. Plasma lipoprotein lipase is also responsible for the clearance of a small amount of chylomicrons in plasma. The triglycerides undergo hydrolysis by an intracellular lipase to form free fatty acids (FFA) and glycerol. The released free fatty acids are carried in the unesterified state in plasma as albumin FFA complex. Many tissues, such as the liver, heart, kidney, muscle, lung, testis, brain, and adipose tissue, can oxidize long-chain fatty acids by beta-oxidation. In this way, the long-chain fatty acids are degraded completely to acetyl-CoA, which can be oxidized to CO_2 and water via the citric acid cycle.

Cholesterol absorption: Cholesterol present in the intestinal wall comes from three sources: The diet, bile, and intestinal secretions and cells. Animal products, especially meat, egg yolk, seafood, and whole-fat dairy products, provide the bulk of dietary cholesterol. A similar esterified cholesterol in the diet is rapidly hydrolyzed in the intestine to free cholesterol and free fatty acids (FFA) by cholesterol esterases in pancreatic and small-intestinal secretions.

Cholesterol is absorbed by the formation of mixed micelles containing unesterified cholesterol, fatty acids, mono-glycerides, phospholipids, and conjugated bile acids. Because of their amphipathic properties, the bile acids are the most important factor affecting micelle formation and, therefore, cholesterol absorption. The cholesterol in the intestine is present in the unesterified (free) form. On average, 30% to 60% of dietary and intestinal cholesterol is absorbed daily. Maximum absorption of cholesterol occurs in the small intestine (middle and terminal ileum).

After its absorption into the mucosal cells, cholesterol is reassembled along with triglycerides, phospholipids, and several specific apoproteins into a large micelle called a chylomicron. One apoprotein apo B-48 is vital to the formation of chylomicrons. Chylomicrons enter the lymphatics, which empty into the thoracic duct, and eventually enter the systemic venous circulation.

BAQ: What are malabsorption and steatorrhea? What are the clinical conditions related to malabsorption of fat digestion and absorption?

Ans: Malabsorption means difficulty in the digestion and absorption of food, It can arise from any defect in the digestion and absorption process. Malabsorption of fats can result from the disturbed flow of bile due to hepatic and post-hepatic conditions disturbed intestinal bacterial flora, damage caused to intestinal mucosa due to an inherent disease, impaired absorption of certain nutrients, hyperacidity leading to a change in alkaline pH in the small intestine, congenital defects in the intestinal membrane transport systems, impaired GI motility, infection, compromised blood flow or lymphatic disorders. The result is either a global impairment of absorption of all nutrients or specific nutrients.

A clinical condition related to malabsorption of fats is known as steatorrhea. The stool of a person suffering from steatorrhea contains high amounts of fats. Diseases such as cystic fibrosis and Crohn's disease also affect the absorption of fats. The management of steatorrhea is complex and to diagnose the root cause, it is necessary to find out the basic and clinical aspects of steatorrhea.

METABOLISM OF LIPIDS

BAQ: Write a note on general lipid metabolism.

Ans: The end products of lipid digestion and absorption are chylomicrons. The chylomicrons are finally deposited either in the liver or in the fat storage depots (adipose tissue), which in health, form about 15% of the body weight. Lipoprotein lipase in the adipose tissue is responsible for the clearance of chylomicrons. Plasma lipoprotein lipase is also responsible for the clearance of a small amount of chylomicrons in plasma. The triglycerides undergo hydrolysis by an intracellular lipase to form free fatty acids (FFA) and glycerol. The released free fatty acids are carried in the unesterified state in plasma as albumin FFA complex. Many tissues, such as the liver, heart, kidney, muscle, lung, testis, brain, and adipose tissue, can oxidize long-chain fatty acids by beta-oxidation. In this way, the long-chain fatty acids are degraded completely to acetyl-CoA, which can be oxidized to CO_2 and water through the citric acid cycle. 14 ATPs are obtained by every oxidation cycle of free fatty acid.

BAQ: Write a note on the biosynthesis of fatty acids.

Ans: The biosynthesis of fatty acids is a multi-step process in the cytoplasm mainly of the liver, kidneys, brain, lungs, mammary gland, and adipose tissues. Glucose provides the primary substrate for lipogenesis. Synthesis of fatty acids requires NADPH coenzyme and an activated two-carbon intermediate, acetyl-CoA. The acetyl-CoA in fat synthesis exists temporarily bound to the enzyme complex as malonyl-CoA.

In fatty acid synthesis, acetyl-CoA is the direct precursor only of the methyl end of the growing fatty acid chain. All the other carbons come from the acetyl group of acetyl-CoA but only after it is modified to provide the actual substrate for fatty acid synthase, malonyl-CoA.

Acetyl-CoA enters the cytoplasm in the form of citrate through the tricarboxylate transport system. In the cytoplasm, citrate is converted to oxaloacetate and acetyl-CoA by the ATP-driven ATP-citrate lyase reaction.

The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction is acetyl-CoA carboxylase (ACC), which requires a biotin co-factor (Fig. 5.8).

The synthesis of fatty acids from acetyl-CoA and malonyl-CoA is carried out by fatty acid synthase (FAS). The active enzyme is a dimer of identical subunits.

All of the reactions of fatty acid synthesis are carried out by the multiple enzymatic activities of FAS. Fatty acid synthesis involves 4 enzymatic activities. These are beta-keto-ACP synthase, beta-keto-ACP reductase, 3-OH acyl-ACP dehydratase, and enoyl-CoA reductase. The two reduction reactions require NADPH oxidation to $NADP^+$.

The primary fatty acid synthesized by FAS is palmitate. It is then released from the enzyme and can then undergo separate elongation and/or unsaturation to yield other fatty acid molecules.

Formation of malonyl-CoA is the commitment step for fatty acid synthesis because malonyl-CoA has no metabolic role other than serving as a precursor to fatty acids.

Fatty acid synthase (FAS) carries out the chain elongation steps of fatty acid biosynthesis.

SAQ: What is the main difference between fatty acid biosynthesis and fatty acid oxidation?

Ans: In fatty acid biosynthesis, cells synthesize free non-essential fatty acids. In beta-oxidation of fatty acids energy is derived in the form of APT molecules (14) for cellular activities.

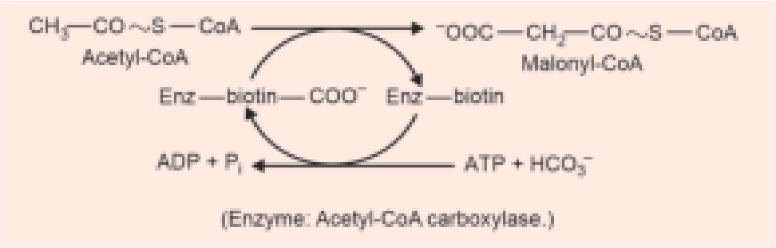


Fig. 5.8: Biosynthesis of malonyl-CoA

The pathway for fatty acid synthesis occurs in the cytoplasm, whereas oxidation occurs in the mitochondria. The other major difference is the use of nucleotide co-factors. Oxidation of fats involves the reduction of $FADH^+$ and NAD^+. Synthesis of fats involves the oxidation of NADPH. However, both oxidation and synthesis of fats utilize an activated two-carbon intermediate, acetyl-CoA.

BAQ: Write a note on the oxidation of fatty acids.

Ans: Oxidation of fatty acids provides energy to the body cells. It takes place when cells require additional energy during fasting, phases between meals, and during strenuous exercises. Oxidation of fatty acids provides the energy requirement of the heart muscle, skeletal muscle, and kidneys when levels of glucose, glycogen, and glucogenic component are depleted. Thus, the oxidation of fatty acids prevents muscles from catabolic breakdown.

Oxidation of fatty acids takes place by alpha-oxidation, beta-oxidation, and omega-oxidation.

Alpha-oxidation is a minor oxidation pathway that occurs in the peroxisomes of a cell. The chain is broken by the carbon chain between C1 and C2 and with the release of carbon dioxide per cycle (Fig. 5.9).

Beta-oxidation is the major mechanism of oxidation of fatty acids and takes place in the mitochondria and also in the peroxisome. Beta oxidation of fatty acids release acetyl-CoA by breaking the carbon chain between C2 and C3 (Fig. 5.9).

Omega-oxidation is a minor oxidation pathway and takes place in the endoplasmic reticulum. The action site for the omega oxidation reaction is the methyl end of the molecule (Fig. 5.9).

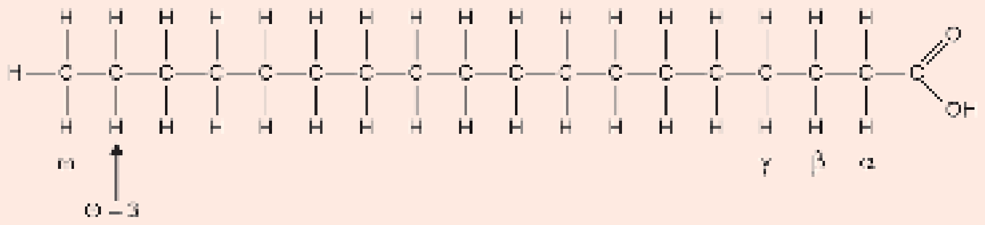
Significance of alpha-oxidation: Simpler forms of fatty acids form during alpha-oxidation of dairy fats like phytanic acid, which later on undergoes beta-oxidation.

Alpha-oxidation also produces intermediate hydroxy fatty acids such as cerebronic acid that can be used for the synthesis of cerebrosides and sulfatides.

Odd chain fatty acids are produced by the decarboxylation of fatty acids that can be used in the synthesis of sphingolipids.

Significance of beta-oxidation of fatty acids: Every beta-oxidation cycle of free fatty acid produces 17 ATP molecules, since NADH produces 3 ATP, FADH2 produces 2 ATP and a full rotation of acetyl-CoA in the citric acid cycle produces 12 ATP.

Significance of omega-oxidation of fatty acids: Omega oxidation serves as a minor but



Use of Greek letters to designate carbons

The carbon next to the –COOH group is designated α; the next one is β, and so forth. The most distant carbon is designated ω. Sometimes carbon atoms close to the ω carbon are designated in relation to it, e.g., the third from the end is ω–3

Fig. 5.9: Structural formula of a fatty acid indicating alpha, beta, gamma, and omega carbons

important alternative pathway to defective β-oxidation pathways. Omega oxidation can form glucose from succinyl-CoA during starvation.

SAQ: What is Refsum disease?

Ans: Refsum disease is caused by the deficiency of the enzyme phytanoyl-CoA 2-hydroxylase which leads to a defect in the alpha-oxidation pathway of the phytanic acid. Thus, phytanic acid is accumulated in cells and tissues with the presentation of symptoms such as peripheral neuropathy, and cerebral degeneration due to neurological damage. Symptoms of Refsum disease include scaly skin, difficulty in hearing, development of cataracts, ataxia, and night blindness.

LAQ: Describe metabolic pathways and energetics of beta-oxidation of fatty acids.

Ans: Fatty acid oxidation is an aerobic process that requires oxygen. In the oxidation process, the chain of free fatty acids is broken between alpha-2 and beta-3 carbon atoms; hence it is called beta-oxidation (Fig. 5.10).

The beta-oxidation of fatty acids starts at the carboxyl end and involves the following three stages: (1) Activation of fatty acids in the cytosol, (2) Transport of fatty acids into mitochondria (carnitine shuttle), and (3) Proper beta oxidation in the mitochondrial matrix.

1. Once in the cytosol, activation of the fatty acid is catalyzed by the enzyme fatty acyl-CoA synthetase (thiokinase). A fatty acid reacts with ATP to give a fatty acyl adenylate and inorganic pyrophosphate, which then reacts with free coenzyme A to give a fatty acyl-CoA ester and AMP. The fatty acyl-CoA then reacts with carnitine to form acylcarnitine, which is transported across the inner mitochondrial membrane by monosodium glutamate.
2. Once inside the mitochondria, each cycle of beta-oxidation, liberates a two-carbon unit-acetyl-CoA, in a sequence of the following four reactions:
   A. Dehydrogenation by FAD: The first step is the oxidation of the fatty acid by Acyl-CoA-dehydrogenase. The enzyme catalyzes the formation of a double bond between the C-2 and C-3. The end product of this reaction is *trans*-2-*trans*-enoyl-CoA and $FADH_2$.
   B. Hydration: The next step is the hydration of the bond between C-2 and C-3. The reaction is stereospecific, forming only the L isomer, and it is catalyzed by enoyl-CoA hydratase. The end product of this reaction is L-beta-hydroxyacyl-CoA.
   C. Oxidation by NAD^+: The third step is the oxidation of L-beta-hydroxy acyl-CoA by NAD^+, which converts the hydroxyl group into a keto group. L-beta-hydroxy acyl-CoA dehydrogenase catalyzes this reaction. The end product of this step is beta-ketoacyl-CoA.
   D. Thiolysis: In this final step, the cleavage of beta-ketoacyl-CoA takes place by the thiol group of another molecule of CoA. The thiol is inserted between C-2 and C-3. This reaction is catalyzed by beta-ketothiolase. The end product of this reaction is an acetyl-CoA molecule and an acyl-CoA molecule that is two carbons shorter.

During this process, the entire chain is cleaved into acetyl-CoA units. The final cycle produces two separate acetyl-CoA molecules.

For every cycle, the acyl-CoA unit is shortened by two carbon atoms. During this process, one molecule of $FADH_2$, NADH, and acetyl-CoA are formed.

Following hormones play an important role in beta-oxidation of fatty acids:

Insulin enhances lipogenesis and increases the oxidation of glucose through hexose monophosphate shunt.

Other hormones, such as adrenocorticotropic hormone (ACTH), growth hormone (GH), thyroid stimulating hormone (TSH), epinephrine, norepinephrine, and glucagon,

β-oxidation of fatty acids

- It is the principal method by which fatty acids are oxidized
- A fatty acid is oxidized step by step by the sequential removal of 2 carbon units
- Coenzyme A is added to the carboxyl group of the fatty acid to form fatty acetyl-CoA derivative
- Each 2-carbon unit is liberated from the fatty acid as acetyl-CoA
- The various enzymes collectively known as "fatty acid oxidase" are found associated with the enzymes of the respiratory chain in mitochondria. These enzymes catalyse the oxidation of fatty acids to acetyl-CoA, which enters citric acid cycle

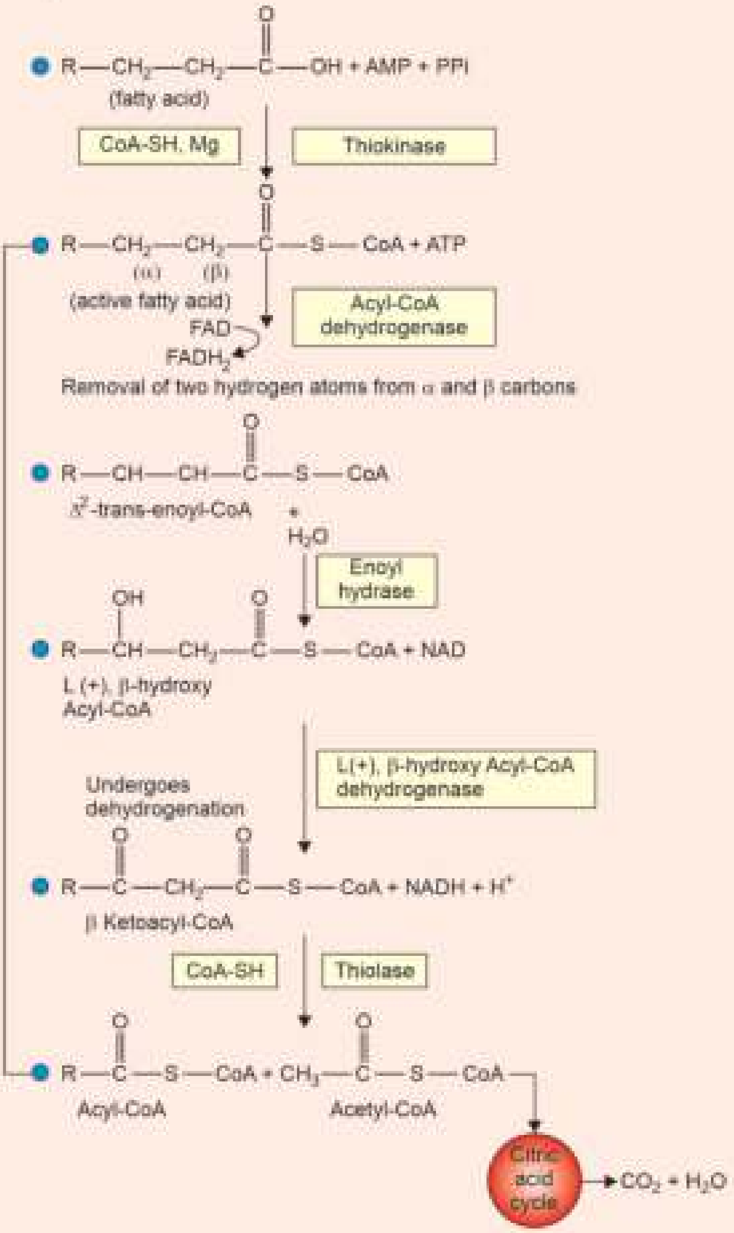

R—CH₂—CH₂—C(=O)—OH + AMP + PPi
(fatty acid)
CoA-SH, Mg
Thiokinase
R—CH₂—CH₂—C(=O)—S—CoA + ATP
(α) (β)
(active fatty acid)
FAD
FADH₂
Acyl-CoA dehydrogenase
Removal of two hydrogen atoms from α and β carbons
R—CH—CH—C(=O)—S—CoA
Δ²-trans-enoyl-CoA
+
H₂O
Enoyl hydrase
OH
R—CH—CH₂—C(=O)—S—CoA + NAD
L (+), β-hydroxy Acyl-CoA
L(+), β-hydroxy Acyl-CoA dehydrogenase
Undergoes dehydrogenation
R—C(=O)—CH₂—C(=O)—S—CoA + NADH + H⁺
β Ketoacyl-CoA
CoA-SH
Thiolase
R—C(=O)—S—CoA + CH₃—C(=O)—S—CoA
Acyl-CoA
Acetyl-CoA
Citric acid cycle
CO₂ + H₂O

Fig. 5.10: Metabolic pathways of beta-oxidation of fatty acids

raise the plasma FFA level by increasing the rate of lipolysis of the triglyceride store.

Every beta-oxidation cycle of free fatty acid produces 14 ATP molecules, since NADH produces 2.5 ATP, $FADH_2$ produces 1.5 ATP and a full rotation of acetyl-CoA in the citric acid cycle produces 10 ATP.

LAQ: Write a note on ketogenesis.

Ans: Ketogenesis is the process by which ketone bodies are produced as a result of fatty acid breakdown. It takes place when there is a high rate of fatty acid oxidation in the liver. Ketone bodies are produced mainly in the mitochondria of liver cells.

Synthesis of ketone bodies takes place in response to low glucose levels in the blood, (during fasting), in diabetes mellitus, when cells cannot use glucose, and after exhaustion of cellular carbohydrate stores, such as glycogen. The production of ketone bodies is then initiated to make available energy that is stored as fatty acids.

Fatty acids are enzymatically broken down in β-oxidation to form acetyl-CoA. Under normal conditions, acetyl-CoA is further oxidized and its energy is transferred as electrons to NADH, $FADH_2$, and GTP in the citric acid cycle (TCA cycle). However, if activity in the TCA cycle is low due to low amounts of intermediates such as oxaloacetate, acetyl-CoA is then used instead in the biosynthesis of ketone bodies via acetoacetyl-CoA and β-hydroxy-β-methylglutaryl-CoA (HMG-CoA). Besides its role in the synthesis of ketone bodies, HMG-CoA is also an intermediate in the synthesis of cholesterol.

The three ketone bodies formed are acetoacetate, acetone, and β-hydroxybutyrate. Acetoacetate, which, if not oxidized to form usable energy, is the source of the two other ketone bodies such as acetone, and β-hydroxybutyrate. The formation of ketone bodies takes place as follows (Fig. 5.11):

- Enzymes responsible for ketone body formation are associated mainly with mitochondria. In β-oxidation, two acetyl-CoA molecules formed condensed with one another to form acetoacetyl-CoA. This reaction is catalyzed by thiolase.
- Condensation of acetoacetyl-CoA with another molecule acetyl-CoA by 3-hydroxy-3-methylglutaryl-CoA synthase forms 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).
- Acetoacetate is liberated from HMG-CoA by the action of 3-hydroxy-3-methylglutaryl-CoA lyase.
- Nonenzymatic decarboxylation leads to the formation of acetone and D-beta-hydroxybutyrate dehydrogenase acts on acetoacetate to form D-beta-hydroxy-butyrate.

Ketone bodies are created at moderate levels during sleep and other times when no carbohydrates are available. However, when ketogenesis takes place at higher than normal levels, the body is said to be in a state of ketosis. Both acetoacetate and beta-hydroxy-butyrate are acidic. If levels of these ketone bodies increase significantly, the pH of the blood drops, resulting in ketoacidosis. Hence ketogenesis is controlled in normal individuals. Ketoacidosis is known to occur in untreated diabetes and alcoholics after prolonged binge-drinking without intake of sufficient carbohydrates.

Control of Ketogenesis

1. Ketogenesis does not occur unless there is an increase in the levels of free fatty acids due to increased lipolysis.
2. After uptake by the liver, free fatty acids are either beta-oxidized, converted into ketone bodies, or esterified to triacylglycerol or phospholipids. Fatty acid entry into oxidative is regulated by carnitine palmitoyltransferase-I (CPT-I), and the remaining fatty acids are esterified. CPT-I activity is low in the fed state, leading to depression of fatty acid oxidation. CPT-I activity is high in starvation, leading to an increase in fatty acid oxidation.

SMQ: What are the reasons for carnitine deficiency? Write the clinical significance of carnitine deficiency.

Ans: Carnitine deficiency can occur mainly in newborns due to inadequate biosynthesis or renal leakage. Losses of carnitine can occur also in hemodialysis. Clinical features of carnitine deficiency are hypoglycemia due to impaired fatty oxidation and lipid accumulation with muscular weakness.

SMQ: What is the significance of carnitine palmitoyltransferase-I (CPT-I)?

Ans: CPT-I deficiency affects the liver, resulting in reduced fatty acid oxidation

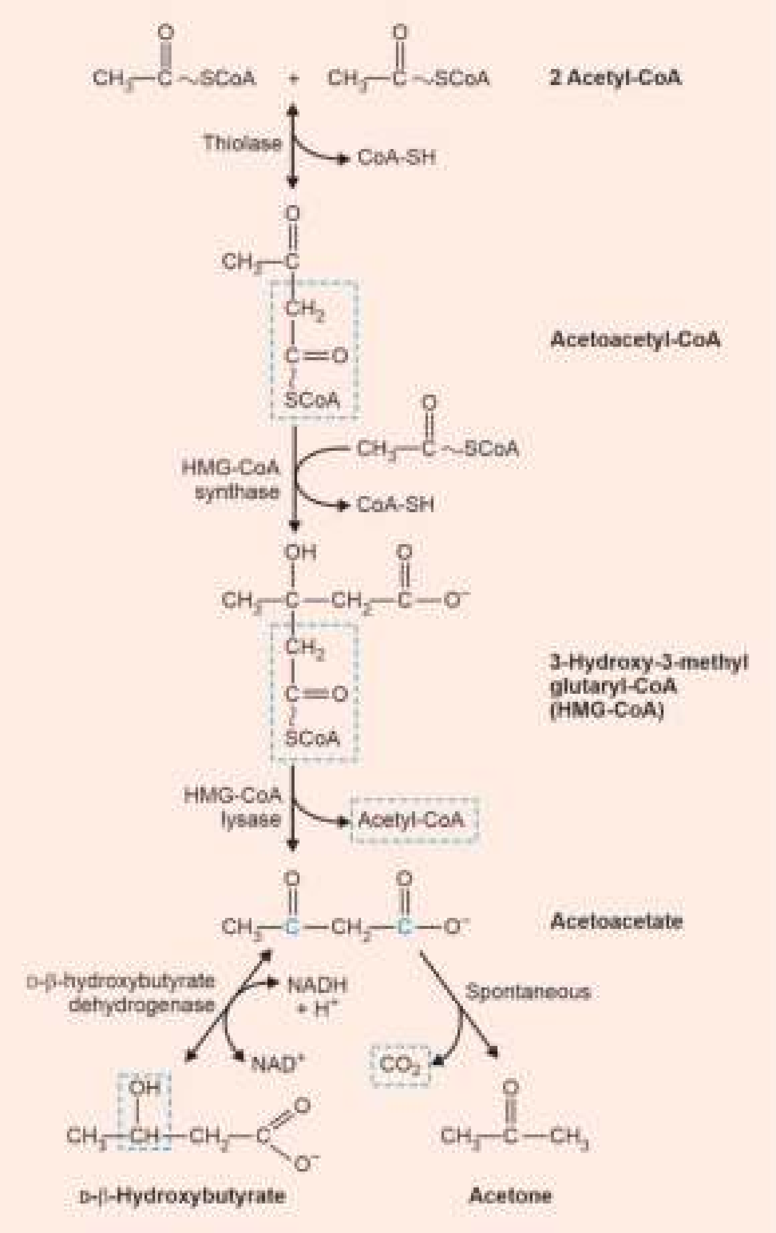

2 Acetyl-CoA
Thiolase
CoA-SH
Acetoacetyl-CoA
HMG-CoA synthase
CoA-SH
3-Hydroxy-3-methyl glutaryl-CoA (HMG-CoA)
HMG-CoA lysase
Acetyl-CoA
Acetoacetate
D-β-hydroxybutyrate dehydrogenase
NADH + H⁺
NAD⁺
Spontaneous
CO₂
D-β-Hydroxybutyrate
Acetone

Fig. 5.11: Reactions of ketogenesis

and ketogenesis with hypoglycemia. CPT-II deficiency affects skeletal muscle and the liver.

SAQ: What is the effect of sulfonylurea drugs such as tolbutamide and glyburide used to treat type II diabetes on carnitine palmitoyl-transferase-I (CPT-I)?

Ans: The sulfonylurea drugs such as tolbutamide and glyburide used to treat type II diabetes are responsible to reduce fatty acid oxidation and hyperglycemia by inhibiting CPT-I.

SAQ: What is Zellweger's syndrome?

Ans: Zellweger's syndrome occurs in individuals due to a rare inherited absence of peroxisomes in all tissues.

The signs and symptoms of Zellweger syndrome are seen in the newborn and include skeletal abnormalities, distinctive facial features, seizures, hearing loss, vision loss, and poor muscle tone (hypotonia).

LAQ: Describe metabolic steps involved in cholesterol synthesis.

Ans: Cholesterol synthesis (Fig. 5.12): Although a portion of the body's cholesterol is derived from dietary intake, most tissue and plasma cholesterol is synthesized endogenously by the liver and other tissues from simpler molecules, particularly acetate.

Although essentially all cells can synthesize cholesterol from acetyl-CoA, almost 90% of synthesis occurs in the liver and gut. Peripheral cells and other organs depend on cholesterol delivery from circulation. Hepatic synthesis of cholesterol appears to be inhibited by newly absorbed cholesterol, which reaches the liver in the chylomicron remnant. This feedback mechanism assists in the control of the cholesterol body pool by adjusting the rate of endogenous synthesis against the rate of dietary absorption.

Cholesterol Biosynthesis

Cholesterol biosynthesis occurs in three following stages: In the first stage, acetyl-CoA molecules derived from carbohydrates, amino acids, and fatty acids are condensed to form the six-carbon thioester HMG-CoA.

In the second stage, HMG-CoA is reduced and decarboxylated to five-carbon isoprene units. These isoprene units are condensed to form first, a 10-carbon and then a 15-carbon intermediate, the latter being farnesyl pyrophosphate.

Two of the C15 molecules combine to produce the final product of the second stage-squalene (a 30-carbon acyclic hydrocarbon).

The second stage is important because it contains the step involving the microsomal enzyme HMG-CoA reductase—the step that is rate limiting in cholesterol biosynthesis.

The third and final stage of synthesis occurs in the endoplasmic reticulum, with many of the intermediate products being bound to a specific carrier protein.

Squalene, after initial oxidation undergoes cyclization to form the 4-ring, 30-carbon intermediate lanosterol.

In a series of oxidation decarboxylation reactions, several side chains are removed from the pentano phenanthrene structure to form the 27-carbon molecule of cholesterol.

Once synthesized, cholesterol is released into circulation for transport in combination with specific apolipoproteins. Lipoproteins transport free cholesterol in the circulation, where it readily equilibrates with cholesterol in other lipoproteins and membranes.

Cholesteryl ester is a storage form of cholesterol found in most tissues. It is transported as cargo in the hydrophobic core of lipoproteins. LDL is the mediator of cholesterol and cholesteryl ester uptake into many tissues.

Free cholesterol is removed from tissues by HDL and transported to the liver for conversion to bile acids in the process known as reverse cholesterol transport. Thyroid hormones play a very important role in the synthesis and degradation of cholesterol and triglycerides.

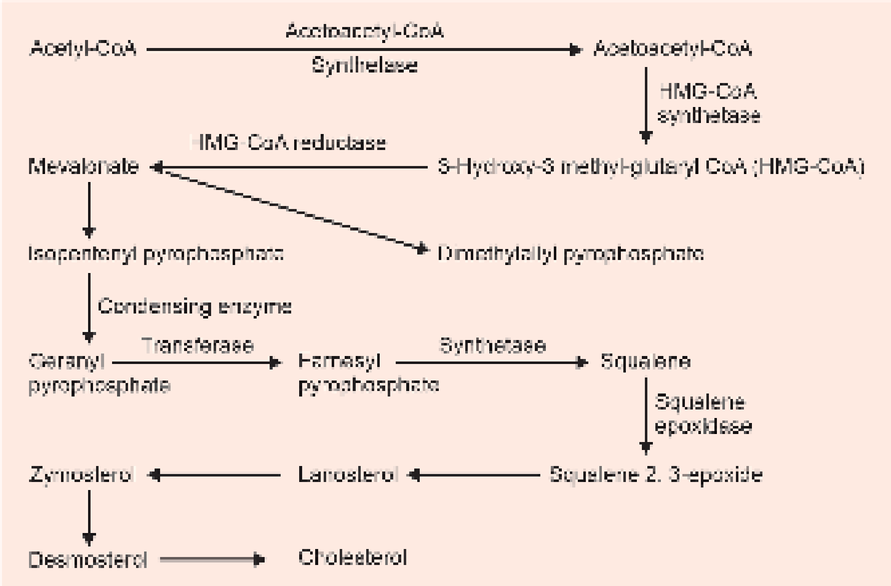

Acetyl-CoA
Acetoacetyl-CoA
Synthetase
Acetoacetyl-CoA
HMG-CoA
synthetase
HMG CoA reductase
Mevalonate
3-Hydroxy-3 methyl-glutaryl CoA (HMG-CoA)
Isopentenyl pyrophosphate
Dimethylallyl pyrophosphate
Condensing enzyme
Transferase
Geranyl
pyrophosphate
Farnesyl
pyrophosphate
Synthetase
Squalene
Squalene
epoxidase
Zymosterol
Lanosterol
Squalene 2, 3-epoxide
Desmosterol
Cholesterol

Fig. 5.12: Cholesterol biosynthesis

CHOLESTEROL ABSORPTION

BAQ: Write a short note on cholesterol absorption.

Ans: Cholesterol present in the intestinal wall comes from three sources: The diet, bile, and intestinal secretions and cells. Animal products, especially meat, egg yolk, seafood, and whole-fat dairy products, provide the bulk of dietary cholesterol. A similar amount of cholesterol is present in the gut from biliary secretion and the turnover of mucosal cells. Practically all cholesterol in the intestine is present in the unesterified (free) form. Esterified cholesterol in the diet is rapidly hydrolyzed in the intestine to free cholesterol and free fatty acids (FFA) by cholesterol esterases in pancreatic and small-intestinal secretions.

Cholesterol is absorbed by the formation of mixed micelles containing unesterified cholesterol, fatty acids, mono-glycerides, phospholipids, and conjugated bile acids. Because of their amphipathic properties, the bile acids are the most important factor affecting micelle formation and, therefore, cholesterol absorption. In the absence of bile acids, digestion and absorption of both cholesterol and triglyceride are severely impaired. The quantity of dietary cholesterol absorbed appears to be dependent on the amount that can be solubilized by micelles. On average, 30% to 60% of dietary and intestinal cholesterol is absorbed daily with increments in dietary cholesterol, and additional cholesterol is absorbed to a maximum of approximately 1 g/d when oral intake reaches 3 g/d. Cholesterol absorption is also affected by the form in which cholesterol enters the digestive system. The crystalline form is absorbed less than cholesterol occurring in a natural state (egg yolk).

Competency achievement: The student should be able to:

BI4.3: Describe the regulation of lipoprotein metabolism and associated disorders

BAQ: What are apolipoproteins? Give examples of their functions.

Ans: Apolipoproteins are protein components of plasma lipoprotein. These are mainly synthesized in the liver and some apoproteins are also synthesized in the small intestine.

The main function of apolipoproteins is, these bind and transport blood lipids to various tissues of the body for metabolism and utilization.

The following are the various types of apolipoproteins:

- Apo A-I, A-II, A-V, Apo B48, B100, Apo C-I, C-II, and C-III
- Apo A-I, B100, and B48 perform assembly and secretion of the lipoprotein
- Apo B100, B 48E, A-I, and A-II maintain the structural integrity of various lipoproteins
- Apo A-I, A-V, C-I, C-II, and C-III, play important role in the activation or inhibitors of specific enzymes of lipoprotein metabolism
- Apo D plays an important role in the process of removal of excess cholesterol from tissues
- Apo E plays an important role in binding to specific receptors.

LIPOPROTEIN METABOLISM

LAQ: Describe reactions of lipoprotein metabolism.

Ans: Metabolism of chylomicrons: Chylomicrons are end products of lipid digestion and absorption. Chylomicrons are responsible for the transportation of dietary fat and are synthesized by and released from the intestinal epithelial cells. The major lipid fraction is triglyceride, which constitutes more than 80% of the total particles by weight.

Chylomicrons contain various apoproteins such as B-48, A-I, A-II, and A-IV. Chylomicrons traverse the lymphatics into the thoracic duct and then enter the main systemic circulation through the jugular vein. Chylomicrons are eventually acted on by many lipoprotein lipases. These enzymes hydrolyze the triglyceride component to mono-glycerol, glycerol, and free fatty acids, which can then be taken up at the cellular level for energy metabolism or for the resynthesis of triglycerides for storage. The apoproteins are transferred to HDL, and the chylomicron remnant is rapidly removed by the liver. The remnants are rapidly internalized by receptor-mediated endocytosis and degraded in the hepatic lysosomes, thus delivering dietary cholesterol to the liver.

Metabolism of VLDL

VLDL is synthesized in and released from the liver. The main component of VLDL is triglycerides. Besides triglyceride, VLDL contains about 10% cholesterol and several apolipoproteins. Apo B-100 is required for the assembly and secretion of VLDL. Lipoprotein lipase (LL) requires Apo C-II for its activity and then LL acts on VLDL, and a short-lived IDL (intermediate density lipoprotein), partly depleted triglycerides, is formed. Apo E determines the continued catabolic process of IDL to LDL for hepatic uptake and degradation.

The factors that increase both the synthesis of triacylglycerol and the secretion of VLDL by the liver include: (1) The fed state rather than the fasting state. (2) The feeding of diets high in carbohydrates (particularly if they contain sucrose and fructose) leading to high rates of lipogenesis and esterification of fatty acids. (3) High levels of circulating fatty acids. (4) Ingestion of ethanol, and (5) The presence of a high concentration of insulin and low concentration of glucagon, which enhances fatty acid synthesis and esterification and inhibits their oxidation. Other hormones accelerate the release of free fatty acids from adipose tissue and raise the plasma-free fatty acid concentration by increasing the rate of lipolysis of the triacylglycerol stores. These include epinephrine, norepinephrine, glucagon, growth hormone, thyroxine, glucocorticoids, and adrenocorticotropic hormone (ACTH).

Metabolism of LDL

In normal circumstances, IDL is further degraded by hepatic lipoprotein lipase to form LDL. LDL catabolism takes place in the liver and the peripheral tissues as follows:

1. LDL interacts with high-affinity receptor sites located in regions of the cell membrane called coated pits.
2. The bound LDL is then internalized by the invagination of these pits into the cell, where the pits pinch off to form endocytotic vesicles.
3. These vesicles fuse with intracellular lysosomes, and the LDL moiety is subjected to a series of hydrolytic degradation by enzyme actions.
4. The esterified cholesterol in LDL is hydrolyzed by lysosomal cholesterol esterase, and the free cholesterol enters the cytoplasm.

The release of free cholesterol is responsible for three regulatory responses that assist in cholesterol homeostasis:

1. Suppression of the rate-limiting enzyme, HMG-CoA reductase, and of new cholesterol synthesis
2. Activation of Acyl-cholesterol acyl transferase (ACAT) activity to esterify excess cholesterol for intracellular storage
3. Modulation of the number of LDL receptors on the plasma membrane to prevent over-accumulation of intracellular cholesterol through the receptor pathway.

Metabolism of high-density lipoproteins (HDL)

HDL metabolism takes place as follows:

- HDL is synthesized and secreted from both the liver and intestine in the discoid form as nascent HDLc (diameter 20–25 nm, density 1.019–1.063). The formation of HDLc is almost exclusively dependent on the synthesis and release of apo AI.
- Apo C and apo E are synthesized in the liver and transferred from liver HDL to intestinal HDL when intestinal HDL enters plasma. HDL acts as a storehouse for apo C and apo E required in the metabolism of chylomicrons and VLDL. HDLc contains phospholipid bilayers containing apo A1, apo AII, apo E, apo CII lecithin, and free cholesterol. Apo E plays an important role in the recognition of receptors on the cell surface.
- The plasma enzyme lecithin cholesterol-acyltransferase (LCAT) and LCAT activator AI bind to the HDLc. LCAT converts surface phospholipids of HDLc into lysolecithin and free cholesterol into esterified cholesterol. With the result, HDLc is converted into HDL3, which accepts excess cholesterol from tissues. This process is known as reverse cholesterol transport.
- Apo D transfers esterified cholesterol from HDL3 to chylomicron remnants and IDL with the result HDL2 formation takes place.
- Lysolecithin is transferred to plasma albumin for further transportation. Several plasma enzymes and proteins, such as phospholipid transfer protein (PLTP) and cholesterol ester transfer proteins (CETP) play important roles in this process.
- Through the chylomicron remnants and LDL, the esterified cholesterol is taken up by the hepatic cells and is used for the synthesis of bile salts.
- HDL3 (5–10 nm, density 1.125–1.210) is reformed mainly by hydrolysis of HDL2, phospholipids, and triglycerides by hepatic lipase. HDL3 then continues to participate in cholesterol efflux from tissues.
- In HDL metabolism, the excess unesterified cholesterol is removed from the circulation, which involves actions of LCAT and apo E.
- HDL may be taken up by hepatocytes by specific receptors. In this process, phospholipids and triglycerides are transferred to hepatocytes without internalizing HDL.
- HDL remains in circulation for several days and eventually may be internalized in the liver.

Q: How many types of HDL function in HDL metabolism? Which type of HDL cholesterol plays an important role in excess cholesterol efflux? What is reverse cholesterol transport?

Ans: HDL-C, HDL-2, and HDL-3 play important roles in HDL metabolism. HDL-3 plays an important part in cholesterol efflux from tissues, mediated by lipoprotein particles containing Apo A-I. This is known as reverse cholesterol transport.

SAQ: What is the clinical significance of an increase in LDL?

Ans: With increasing plasma levels of LDL, the macrophages (scavenger cells) take up larger amounts of the circulating lipoprotein for degradation. A receptor on the macrophages rapidly binds and internalizes an oxidized form of LDL. Oxidative modification of LDL can occur by several possible mechanisms, accelerating the potential for atherosclerosis. At some point, the macrophages become overloaded with cholesterol esters and take on the appearance of foam cells. Foam cells, therefore, become the hallmark of atherosclerotic plaque. In the arterial wall, both macrophages and smooth muscle accumulate cholesterol esters by this mechanism.

SAQ: Enumerate functions of the liver in lipid metabolism.

Ans: Following are the functions of the liver in lipid metabolism:

1. Synthesis and oxidation of fatty acids
2. Synthesis of triglycerides
3. Synthesis of cholesterol and its derivative, such as bile salts
4. Synthesis of phospholipids
5. Synthesis of VLDL and HDL (plasma lipoproteins)
6. Formation of ketone bodies

SAQ: What is cholesterol ester storage disease and related symptoms?

Ans: When esterified cholesterol enters a cell the esters are hydrolyzed by the action of specific esterases present in lysosomes. The lack or malfunction of lysosomal esterases due to genetic error results in intracellular accumulation of cholesterol esters and produces a clinical disorder known as cholesterol ester storage disease. In this disorder, LDL level increases with a decrease in HDL level, leading to atherogenic risk.

BAQ: What is dyslipidemia? What is the clinical significance of dyslipidemia?

Ans: Dyslipidemia means an abnormal change in the normal composition of various normal lipoproteins such as chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL). Dyslipidemia occurs due to primary reasons (primary dyslipoproteinemia) or secondary reasons (secondary dyslipoproteinemia).

Dyslipidemia is most clearly associated with an increased risk for cardiovascular disease (CAD) due to hypercholesterolemia. Elevated plasma levels of cholesterol are carried in LDL. LDL contains approximately 70 percent of the cholesterol in the blood and is the primary target of intervention in the guidelines of the treatment panel of the National Cholesterol Education Program (NCEP), USA.

SAQ: What are primary dyslipoproteinemias?

Ans: A few individuals in the population exhibit inherited defects in lipoprotein metabolism. These conditions are caused by a defect at a stage in the course of lipoprotein formation, transport, or degradation.

The inherited disorders of lipid transport compose a heterogeneous group of syndromes that are most frequently classified according to abnormal concentrations of one or more classes of lipoproteins in plasma. In primary dyslipoproteinemia, increased levels of the following lipoproteins are seen: Chylomicrons, LDL, or VLDL, or an increase in all these lipoproteins.

SAQ: What is secondary dyslipoproteinemias (acquired hyperlipidemia)?

Ans: Secondary dyslipoproteinemias result from some underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism. They manifest as an increase in plasma triglyceride levels, an increase in plasma cholesterol levels, or an increase in both plasma cholesterol and triglycerides. The plasma HDL may be normal or decreased. Hyperlipidemia may be due to elevated circulating LDL and VLDL. Secondary Dyslipoproteinemias could be treated well if the underlying disorder is treated first. The secondary dyslipoproteinemia is observed in the following clinical conditions: Diabetes mellitus, kidney diseases, liver diseases, hypothyroidism, and in alcoholism.

BAQ: What is the primary goal in the treatment of hypercholesterolemia?

Ans: The primary goal of therapy in the treatment of hyperlipidemia is to reduce the plasma concentrations of known atherogenic lipoproteins, thereby reducing or even reversing the flux of lipids from plasma into the arterial wall.

The therapeutic interventions directed at modifying plasma concentrations of lipids and lipoproteins in patients identified to have hyperlipidemia have the following two goals:

1. To reduce the plasma concentrations of known atherogenic lipoproteins, particularly LDL, VLDL, and remnants and concurrently to increase plasma concentrations of potentially antiatherogenic HDL, therefore exerting a favorable effect upon lipid deposition in the arterial wall.
2. To decrease the plasma concentrations of triglyceride-rich lipoproteins, i.e. chylomicrons and VLDL, in patients with severe hypertriglyceridemia, thereby preventing the development of hepatomegaly, splenomegaly, eruptive xanthomas, and pancreatitis as well as to reduce the long-term risk of atherosclerosis.

Determination of the plasma concentrations of cholesterol and triglycerides with concurrent measurement of HDL cholesterol represents the basic lipid profile necessary for the diagnosis of most hyperlipidemia.

Recent expert panels in the United States, Europe, Great Britain, and Canada have defined specific cut-off points for the diagnosis of hypercholesterolemia in adults and have suggested levels of LDL cholesterol above which diet and drug therapy should be considered. Under optimal circumstances, treatment decisions should be based on lipid and lipoprotein concentrations.

BAQ: What are the standard guidelines given by Cholesterol Education Programs?

Ans: The National Cholesterol Education Program (NCEP) is a program managed by the National Heart, Lung, and Blood Institute, USA. The goal of NCEP is to reduce increased cardiovascular disease rates due to hypercholesterolemia (elevated cholesterol levels) in the United States of America and provide guidelines for the world population. The report of NCEP classifies people according to total serum cholesterol levels. Serum cholesterol of 200 mg/dl or less is called desirable blood cholesterol, 200 to 239 mg/dl is considered borderline high-blood cholesterol, and 240 mg/dl and above is classified as high serum cholesterol.

NCEP has recommended aggressive drug and dietary therapy for persons with cardiovascular risk-factors. It is necessary to keep serum LDL-cholesterol below 130 mg/dl for patients with cardiovascular risk factors.

The National Cholesterol Education Program (NCEP) panel has advocated a more aggressive approach to lipid-lowering therapy with evidence of atherosclerosis or those who have two other cardiovascular risk factors. In such patients, drug therapy should be considered when the LDL cholesterol concentrations exceed 160 mg/dl on maximal dietary therapy. In patients without evidence of atherosclerosis, the NCEP panel advocated a therapeutic goal of under 160 mg/dl for LDL cholesterol, whereas for individuals with atherosclerosis or the concurrent presence of two risk factors, a lower level of LDL less than 130 mg/dl is desirable. Concentrations of LDL cholesterol below 95 mg/dl may be necessary for the regression of atherosclerosis in humans.

SAQ: Enumerate the factors considered in the decision to use drugs to reduce total and LDL-cholesterol.

Ans: Factors to be considered in the decision to use drugs to reduce LDL-cholesterol concentrations include:

1. The magnitude, duration, and etiology of hypercholesterolemia
2. The family history of premature coronary artery disease
3. The age and sex of the patient
4. The presence or absence of atherosclerosis
5. The concurrent presence of other risk factors for coronary artery disease (e.g. low levels of HDL, diabetes), the attitude of the patient towards drug therapy, and the potential benefits to be derived from such treatment.

BAQ: What are the various types of drugs used to treat dyslipidemia?

Ans: The following are the various types of drugs used to treat dyslipidemia:

Statins: Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays an important role in the production of cholesterol in the liver. Statins are particularly suitable for lowering LDL. These drugs lower LDL-C by 18% to 55%, depending on the specific statin being used. Statins inhibit the rate-limiting enzyme HMG-CoA reductase in cholesterol synthesis and decrease blood cholesterol levels.

The bile acid sequestrants

The bile acid sequestrants are a group of drugs used to bind certain components of bile in the gastrointestinal tract. They disrupt the enterohepatic circulation of bile acids by sequestering them and preventing their reabsorption from the gut. Bile acid sequestrants (resins, e.g. cholestyramine) are particularly effective for decreasing LDL-C by facilitating the excretion of cholesterol-containing bile acids into the intestine and preventing their reabsorption from the intestine. It decreases LDL by 15–30% and raises HDL by 3–5%. Bile acid sequestrants may cause gastrointestinal disturbances and may also reduce the absorption of other drugs and vitamins from the gut.

Fibrates: Fibrates are indicated for treating hypertriglyceridemia. Their action is exerted by increasing the activity of lipoprotein lipase, which decreases circulating triglycerides by hydrolyzing them into glycerol and free fatty acids. Fibrates specifically decrease triglycerides by 20–50% with an increase in the level of HDL.

Niacin: Niacin blocks the breakdown of fats in adipose tissue, which is used to build very low-density lipoproteins (VLDL) in the liver. VLDL are precursors of low-density lipoprotein (LDL). Because niacin blocks the breakdown of fats, it causes a decrease in free fatty acids in the blood and, as a consequence, decreases the secretion of VLDL and cholesterol by the liver. Niacin also lowers triglycerides by 20–50%. It may also lower LDL by 5–25% and increase HDL by 15–35%.

Ezetimibe (Zetia): Ezetimibe is used to decrease circulating blood cholesterol. It is a selective inhibitor of dietary cholesterol absorption.

Orlistat (Xenical): Orlistat is a drug used to treat obesity. It prevents the absorption of about 30% of fats from the human diet and reduces caloric intake. It inhibits pancreatic lipase that breaks down triglycerides in the intestine.

SAQ: What are the common side effects of cholesterol-lowering drugs?

Ans: The following are common side effects of cholesterol-lowering drugs: Nausea, diarrhea, constipation, pain, weakness, stomach cramps, muscle soreness, vomiting, fatigue, headache, dizziness, drowsiness, rash, flushed skin, etc.

Statins are common cholesterol-lowering drugs. However, in some patients, they may cause severe side effects like liver damage, muscle damage, dark-colored urine, memory loss, and confusion.

Multiple Choice Questions

Q1. Anti-inflammatory corticosteroids inhibit the synthesis of

A. Prostaglandins
B. Leukotrienes
C. Thromboxanes
D. All of these
E. Only B and C

Q2. Diets having a high ratio of polyunsaturated: saturated fatty acids can cause

A. Increase in serum LDL
B. Increase in serum VLDL
C. Increase in serum HDL
D. Increase in serum triglycerides

Q3. Thromboxanes cause

A. Platelet aggregation
B. Vasodilation
C. Platelet destruction
D. All of these

Q4. Sphingosine is synthesized from

A. Acetyl-CoA and choline
B. Palmitoyl-CoA and ethanolamine
C. Palmitoyl-CoA and choline
D. Palmitoyl-CoA and serine

Q5. One of the coenzymes required for the synthesis of triglycerides

A. Pyridoxin
B. Flavin
C. Pyridoxal phosphate
D. NADPH

Q6. Cerebrosides contain all the following *except*

A. Galactose
B. Phosphate
C. Sphingosine
D. Fatty acid

Q7. Chylomicron remnants are catabolized in

A. Adipose tissue
B. Liver
C. Intestine
D. All of the above

Q8. VLDL remnant may be converted into

A. HDL
B. VLDL
C. LDL
D. Chylomicrons

Q9. Receptors for chylomicron remnants require

A. Apo B-48
B. Apo CI
C. Apo D
D. Apo A

Q10. LDL receptors are specific for

A. Apo B-48
B. Apo B-100
C. Apo D
D. B and C

Q11. HDL is synthesized in

A. Liver
B. Adipose tissue
C. Intestine
D. A and C

Q12. Activated lecithin cholesterol acyltransferase is essential for the conversion of

A. HDL2 into HDL3
B. VLDL remnants into LDL
C. Nascent HDL into HDL3
D. HDL3 into HDL2

Q13. Lipid stores are present mainly in

A. Muscles
B. Adipose tissue
C. Liver
D. Brain

Q14. In adipose tissue, glycerol 3-phosphate required for the synthesis of triglycerides is derived mainly from:

A. Hydrolysis of phospholipids
B. Hydrolysis of pre-existing triglycerides
C. Glycerol
D. Dihydroxyacetone phosphate formed in glycolysis

Q15. Glycerol released from adipose tissue by hydrolysis of triglycerides is mainly

A. Taken up by brain
B. Taken up by red blood cells
C. Taken up by liver
D. Taken up by kidneys

Q16. Free glycerol cannot be used for triglyceride synthesis in the

A. Brain
B. Kidney
C. Adipose tissue
D. Liver

Q17. Adipose tissue lacks this enzyme

A. Glycerol kinase
B. Lipoprotein lipase
C. Hormone-sensitive lipase
D. Glycerol 3-phosphate dehydrogenase

Q18. Pancreatic lipase requires for its activity

A. Phospholipids
B. Bile salts
C. Co-lipase
D. A, B and C

Q19. Oxidation of fatty acids occurs

A. On the cytosol
B. On the inner mitochondrial membrane
C. In the matrix of mitochondria
D. In the nucleus

Q20. In β-oxidation activation of fatty acids requires the enzyme

A. Thiokinase
B. Coenzyme A
C. Lipoprotein lipase
D. Thiolase
E. A and D
F. A, B, and C

Q21. Mitochondrial thiokinase acts on

A. Long-chain fatty acids
B. Short-chain fatty acids
C. Unsaturated fatty acids
D. Saturated fatty acids

Q22.Carnitine is required for the transport of

A. Long-chain fatty acids into mitochondria
B. Triglycerides into mitochondria
C. Short chain fatty acids into mitochondria
D. Chylomicrons into the cytosol

Q23. Net ATP generated by the complete oxidation of stearic acid is

A. 4
B. 36
C. 106
D. 146

Q24. β-Oxidation of fatty acids occurs mainly in

A. Muscles
B. Liver
C. Brain
D. Adipose tissue

Q25. Gaucher's disease is due to the deficiency of the enzyme

A. Sphingomyelinase
B. Galactocerbrosidase
C. Glucocerebrosidase
D. β-Galactosidase

Q26. Characteristic finding in Gaucher's disease is

A. Skeletal disorders
B. Neurological disorders
C. Hepatosplenomegaly
D. All of the above

Q27. Fucosidosis is characterized by

A. Muscle spasticity
B. Liver enlargement
C. Mental retardation
D. A, B, and C

Q28. Metachromatic leukodystrophy is due to a deficiency of the enzyme

A. Ceramidase
B. Hexosaminidase A
C. α-Fucosidase
D. Arylsulphatase A

Q29. A significant feature of Tangier disease is a significant decrease in

A. HDL
B. LDL
C. VLDL
D. B and C

Q30. In broad beta disease increase in the following lipoproteins is observed

A. Chylomicrons
B. LDL
C. VLDL
D. B and C

Q31. Obesity may cause

A. Non-alcoholic steatohepatitis
B. Non-insulin-dependent diabetes mellitus
C. Increased glucose tolerance
D. A and B

Q32. Atherosclerosis is associated with a diet containing

A. High in unsaturated fatty acids
B. High in saturated fatty acids
C. High in cholesterol
D. B and C

Q33. Refsum's disease results from a defect in the following pathway

A. Omega-oxidation of fatty acids
B. Beta-oxidation of fatty acids
C. Gamma-oxidation of fatty acids
D. Alpha-oxidation of fatty acids

Q34. Salivary lipase is secreted by

A. Dorsal surface of the tongue
B. Parotid glands
C. Sub-maxillary glands
D. A and C

Q35. Co-lipase is a

A. Protein
B. Vitamin A
C. Glycoprotein
D. Phospholipid

Q36. Mitochondrial membrane is permeable to

A. Long-chain fatty acids
B. Medium-chain fatty acids
C. Short chain fatty acids
D. A, B, and C

Q37. During each cycle of β-oxidation

A. Four carbon atoms are removed from the carboxyl end of the fatty acid
B. Two carbon atoms are removed from the methyl end of the fatty acid
C. One carbon atom is removed from the carboxyl end of the fatty acid
D. Three carbon atoms are removed from the methyl end of the fatty acid

Q38. Complete oxidation of palmitic acid leads to the formation of

A. 24 ATPs B. 148 ATPs
C. 106 ATPs D. 116 ATPs

Q39. Extramitochondrial synthesis of fatty acids occurs in

A. Brain
B. Lungs
C. Mammary glands
D. A, B and C

Q40. NADPH required for fatty acid synthesis can be obtained from

A. Oxidative decarboxylation of malate
B. Hexose monophosphate shunt
C. Extramitochondrial oxidation of isocitrate
D. A, B, and C

Q41. Lipooxygenase is required for the synthesis of

A. Thromboxanes B. Prostaglandins
C. Leukotrienes D. Both A and B

Q42. Release of free fatty acids from adipose tissue is not increased by

A. Growth hormone
B. Epinephrine
C. Insulin
D. Glucagon

Q43. Hypocholesterolemic drug Lovastatin is a

A. Competitive inhibitor of HMG-CoA synthetase
B. Competitive inhibitor of acetyl-CoA carboxylase
C. Competitive inhibitor of HMG-CoA reductase
D. A, B, and C

Q44. Abetalipoproteinemia occurs due to a block in the synthesis of

A. Apoprotein B B. Apoprotein AI
C. Apoprotein CII D. Apoprotein D

Q45. Tangier disease is characterized by

A. Decrease in serum HDL
B. Decrease in VLDL
C. Its inheritance as autosomal recessive
D. A and C
E. A, B, and C

Q46. For extramitochondrial fatty acid synthesis, acetyl-CoA may be obtained from

A. Oxaloacetate B. Succinate
C. Citric acid D. Isocitrate

Q47. Fluidity of cell membrane is increased by the following constituent *except*

A. Saturated fatty acids
B. Cholesterol
C. Integral proteins
D. Polyunsaturated fatty acids

Q48. Transition temperature of membranes may be affected by the following constituent of membranes

A. Integral proteins
B. Peripheral proteins
C. Mucopolysaccharides
D. Cholesterol

Q49. Acetyl-CoA formed from pyruvate can be used for the synthesis of all the following *except*

A. Cholesterol B. Glucose
C. Fatty acids D. Steroid hormones

Q50. Which of the following is the main ketone body used as a source of energy in extra-hepatic tissues?

A. Acetone
B. Acetoacetate
C. β-hydroxybutyric acid
D. Oxaloacetate

Q51. Anti-inflammatory corticosteroids inhibit

A. Cyclo-oxygenase
B. Phospholipase A1
C. Phospholipase A2
D. Lipo-oxygenase

Q52. Cyclo-oxygenase is involved in the synthesis of

A. Thromboxanes B. Prostacyclins
C. Prostaglandins D. All of these

Q53. Leukotrienes cause

A. Platelet degradation
B. Increase in capillary permeability
C. Aggregation of platelets
D. A, B and C

Q54. Prostaglandins decrease all of the following *except*

A. Platelet aggregation
B. Blood pressure
C. Blood cholesterol
D. Gastric acid secretion

Q55. Serum cholesterol levels may increase in all of the following *except*

A. Hypothyroidism
B. Hyperthyroidism
C. Nephrotic syndrome
D. Diabetes mellitus

Q56. Free fatty acids released from adipose tissue are transported in the blood by

A. Gamma globulin B. Albumin
C. Beta globulin D. A, B and C

Q57. Deficiency of galactocerebrosidase is observed in

A. Gaucher's disease
B. Fabry's disease
C. Krabbe's disease
D. Metachromatic leukodystrophy

Q58. Deficiency of hexosaminidase A is observed in

A. Fabry's disease
B. Niemann-Pick disease
C. Gaucher's disease
D. Tay-Sachs disease

Q59. Mental retardation occurs in

A. Tay-Sachs disease
B. Gaucher's disease
C. Niemann-Pick disease
D. A, B, and C

Q60. The enzyme deficient in Fabry's disease is

A. α-Galactosidase B. β-Galactosidase
C. α-Glucosidase D. β-Glucosidase

Q61. The concentration of sphingomyelins is increased in

A. Tay-Sachs disease
B. Gaucher's disease
C. Fabry's disease
D. Niemann-Pick disease

Q62. Gaucher's disease is characterized especially by the increase in

A. Butyric acid
B. Glucocerebroside
C. Lignoceric acid
D. Hydroxynervonic acid

Q63. Serum very low-density lipoproteins are separated by electrophoresis as

A. α-lipoproteins
B. β-lipoproteins
C. Pre-β-lipoproteins
D. None of these

Q64. The prostaglandins are synthesized from

A. Oleic acid B. Linoleic acid
C. Arachidonic acid D. Linolenic acid

Q65. These ions are required for the activation of long-chain fatty acids for the enzyme thiokinase

A. Mn^{++} B. Cu^{++}
C. Mg^{++} D. Ca^{++}

Q66. ω-oxidation takes place by the hydroxylase in microsomes, which involves

A. Cytochrome c
B. Cytochrome P-450
C. Cytochrome b
D. Cytochrome a3

Q67. Fatty acids are activated to acyl-CoA by the enzyme thiokinase in the presence of

A. FAD^{+} B. NADP^{+}
C. NADH D. CoA

Q68. Prostaglandins are liberated in the circulation by the stimulation of

A. Parathyroid gland
B. Thyroid gland
C. Adrenal gland
D. Anterior pituitary glands

Q69. Cholesterol is the precursor for the biosynthesis of

A. Bile pigments B. Bile acids
C. Prostaglandins D. Sphingomyelin

Q70. Ketonuria is observed in

A. Diabetes mellitus
B. Diabetes insipidus
C. Prolonged starvation
D. A and C

Q71. Ketone bodies are formed in

A. Brain B. Liver
C. Heart D. Intestines

Q72. Decreases in which of the following HDLs are mainly responsible for the progression of atherosclerosis?

A. HDL3 B. HDLc
C. HDL2 D. A and B

Q73. Mitochondrial lipogenesis requires

A. NADPH
B. Biotin
C. Acetyl-CoA carboxylase
D. NAD

Q74. Fatty acids having a chain length of 10 carbon atoms enter the

A. Systemic circulation
B. Lacteals
C. Portal circulation
D. A, B and C

Q75. Which of the following fatty acid is *not* synthesized in man?

A. Palmitic acid B. Linolenic
C. Butyric acid D. Stearic

Q76. Which of the following lipoproteins is responsible for reverse cholesterol transport from the body?

A. LDL B. HDL
C. VLDL D. Chylomicrons

Q77. The level of free fatty acids in plasma is increased by the action of

A. Sucrose B. Glucose
C. Caffeine D. Insulin

Q78. Lysolecithin is formed from lecithin by the action of

A. Phospholipase D
B. Phospholipase A1
C. Phospholipase C
D. Phospholipase A2

Q79. Cholesterol circulates in the bloodstream mainly as

A. Free cholesterol
B. Ester cholesterol
C. LDL
D. HDL

Q80. Very low-density lipoproteins are relatively rich in

A. Cholesterol B. Phospholipids
C. Free fatty acids D. Triglycerides

Q81. The 'Committed step' in the biosynthesis of cholesterol from acetyl-CoA is

A. Formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA
B. Formation of squalene by squalene synthetase
C. Formation of acetoacetyl-CoA from acetyl-CoA
D. Formation of mevalonate from HMG-CoA

Q82. During a routine examination of the blood of a 15-year-old boy, his serum total cholesterol was 565 mg/dl (Normal range 150– 250 mg/dl) and LDL cholesterol was 385 mg/dl (normal values 130 mg <). He was not diabetic and his renal profile test reports were normal. The probable diagnosis is

A. A case of familial hyperlipidemia
B. Hyperlipidemia due to lifestyle changes
C. A case of syndrome X
D. Both B and C.

Q83. The lipid profile reports of a 45-year-old obese female indicated very high serum total cholesterol, 386 mg/dl (Normal range 150–250 mg/dl) and LDL-cholesterol was 255 mg/dl (normal values 130 mg <). She was also suffering from hypothyroidism.

The probable diagnosis is

A. A case of familial hyperlipidemia
B. Hyperlipidemia due to lifestyle change
C. Secondary hyperlipidemia
D. Both B and C.

Answers

Reasons are given to Case study related MCQs

1. D **2.** C **3.** A **4.** D **5.** D **6.** B **7.** B **8.** C **9.** A **10.** D
11. D **12.** C **13.** B **14.** D **15.** C **16.** C **17.** A **18.** D **19.** C **20.** A
21. B **22.** A **23.** D **24.** C **25.** D **26.** D **27.** D **28.** D **29.** A **30.** D
31. D **32.** D **33.** D **34.** A **35.** A **36.** C **37.** B **38.** C **39.** D **40.** D
41. C **42.** C **43.** C **44.** A **45.** D **46.** C **47.** C **48.** D **49.** D **50.** B
51. B **52.** C **53.** B **54.** C **55.** B **56.** B **57.** C **58.** D **59.** D **60.** B
61. D **62.** B **63.** C **64.** C **65.** C **66.** B **67.** D **68.** C **69.** B **70.** D
71. B **72.** A **73.** A **74.** C **75.** B **76.** B **77.** C **78.** D **79.** C **80.** D
81. D

82. A: Primary dyslipidemia (familial disorder, a genetic disorder). He is only 14 years old and not suffering from any secondary dyslipidemia-related disorder such as diabetes mellitus or nephritis.

83. D: Lifestyle change related to dyslipidemia (since she is obese) and due to hypothyroidism (secondary dyslipidemia), which is not well controlled.

Case Studies

Case 1: In the routine blood examination of a 42-year-old obese male executive, his lipid profile test results were as follows:

	Reference values (Normal values)
Blood glucose fasting: 105 mg/dl	70–110 mg/dl
Serum total cholesterol: 322 mg/dl	150–250 mg/dl
Serum triglycerides: 288 mg/dl	10–190 mg/dl
Serum LDL-cholesterol: 186 mg/dl	< 130 mg/dl
Serum HDL-cholesterol: 40 mg/dl	40–65 mg/dl

1. What is the probable diagnosis?

Ans: Hyperlipidemia, probably due to obesity related to a changed lifestyle. He is not diabetic according to his fasting blood glucose value, hence hyperlipidemia is not due to diabetes mellitus.

2. What is the mechanism behind the increase in serum lipids?

Ans: Obesity, probably due to lack of exercise and may be due to his habit of frequently eating outdoor lipid-rich foods.

3. Why increase in serum triglycerides was seen?

Ans: This may be due to his food and drink habits. Increased intake of sweets and fructose leads to an increase in serum triglycerides. Similarly, if frequent intake of soft drinks is there, then soft drinks contain high fructose corn syrup, which gives rise to serum triglyceride levels.

4. What is the probable line of treatment?

Ans: Statins are usually prescribed as cholesterol-lowering drugs along with a change in lifestyle with regular exercises, a diet low in lipids, and a controlled intake of sweets and soft drinks.

BAQ: Show horizontal integration of symptoms and test reports of Case 1 with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Probability of thickening of arteries due to atherosclerosis.

Horizontal integration with physiology

Increased circulation of free fatty acids, total cholesterol, triglycerides, and HDL-cholesterol

Horizontal integration with nutrition

The suggestion of a diet low in high glycemic sugars like glucose, sucrose, maltose, and fructose (all present in table sugar, sweets, soft drinks, cakes, pastries, sweet fruits, etc.)

BAQ: Shows vertical integration of symptoms and test reports of Case 1 with pharmacology and preventive medicine and pathology subjects

Ans: Vertical integration

Pharmacology: Study of an appropriate drug to treat dyslipidemia.

Preventive medicine: Preventive measures to control normal body mass index (BMI) to prevent insulin resistance and appropriate intake of diet and use of exercises.

Pathology: Study of causes of obesity and dyslipidemia related to lifestyle changes, diagnosis and prognosis.

Case 2: A 34-year-old obese diabetic female complained of a lack of energy, numbness on her face, arms, and feet, lack of concentration, and increased frequency of urination. She was advised by her physician to test her blood specimen for "lipid profile tests". The test reports were as follows:

	Reference range (Normal values)
Blood glucose fasting: 235 mg/dl	70–110 mg/dl
Serum total cholesterol: 290 mg/dl	150–250 mg/dl
Serum LDL-cholesterol: 175 mg/dl	<130 mg/dl
Serum HDL-cholesterol: 45 mg/dl	40–65 mg/dl

1. What is the probable diagnosis?

Ans: Symptoms and blood test reports indicate that she was suffering from dyslipidemia, due to diabetes mellitus (as her fasting blood glucose level was high, >126 mg/dl). Hence, it is a case of secondary dyslipidemia, due to diabetes mellitus.

2. What is the biochemical basis behind the increase in serum total cholesterol and LDL cholesterol?

Ans: In diabetes mellitus, since body cells are unable to use blood glucose for energy, excessive free fatty acids (FFA) are used. The intermediate component of FFA metabolism is acetyl-CoA, which is partly converted for the synthesis of cholesterol. LDL is a vehicle to carry cholesterol, hence there is an increase in LDL-cholesterol.

3. What is the probable line of treatment?

Ans: It is necessary to maintain normal blood glucose by using the appropriate drugs suggested by her physician. It is also necessary to decrease weight by routine exercises. Similarly, a lipid-free and sugar-free diet is appropriate to control serum cholesterol and triglycerides.

Case 3: A 15-year-old boy was referred by his family physician to a dermatologist because of the appearance of extensive yellowish papules, with erythematous bases on his elbows. He was advised to test blood for lipid profile tests, and the report was as follows:

	Reference range (Normal values)
Blood glucose fasting: 105 mg/dl	70–110 mg/dl
Serum total cholesterol: 734 mg/dl	150–250 mg/dl
Serum triglycerides: 180 mg/dl	10–190 mg/dl
Serum LDL cholesterol: 610 mg/dl	<130 mg/dl
Serum HDL cholesterol: 88 mg/dl	40–65 mg/dl

1. What is the probable diagnosis?

Ans: He is not diabetic. From his age, it seems that he is suffering from dyslipidemia, due to a genetic disorder (familial disorder) responsible for primary dyslipidemia.

2. What is the biochemical basis behind the increase in serum total cholesterol and LDL-cholesterol?

Ans: Due to congenital deficiency of enzymes and proteins (apo-proteins) cholesterol metabolism and transportation were affected.

3. Why his skin was affected?

Ans: Papule and erythematous bases seen on the skin were due to the deposition of excess serum cholesterol in the skin.

4. What is the probable line of treatment?

Ans: Statins are prescribed to decrease cholesterol synthesis by the cells with low intake of lipids in their diet, backed by regular exercises.

BAQ: Show horizontal integration of symptoms and laboratory test values of Case 3 with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Affected skin

Horizontal integration with physiology

Increased circulation of total cholesterol and LDL cholesterol

Horizontal integration with nutrition

A suggestion of a diet with a low intake of lipids and a diet low on high glycemic sugars like glucose, sucrose, maltose, and fructose (in table sugar, sweets, soft drinks, cakes, pastries, sweet fruits, etc.)

BAQ: Show vertical integration of symptoms and test reports of Case 3 with pharmacology and preventive medicine and pathology subjects

Ans: Vertical integration:

Pharmacology: Use of appropriate drug to treat primary hypercholesterolemia.

Preventive medicine: Preventive measures like a diet low in lipids and high glycemic sugars. Regular exercises to control normal body mass index (BMI).

Pathology: Study of causes of primary dyslipidemia, diagnosis and prognosis.

Case 4: A 45-year-old obese man was admitted to the hospital with chest pain which had developed after he tried to shift a heavy table. His ECG findings were not normal. He was monitored in the acute coronary unit. His laboratory test results were as follows:

	Normal values
Blood glucose fasting: 103 mg/dl	70–110 mg/dl
Serum total cholesterol: 334 mg/dl	150–250 mg/dl
Serum LDL-cholesterol: 210 mg/dl	<130 mg/dl

1. What is the probable diagnosis?

Ans: He is not diabetic (since his fasting blood glucose was < 110 mg/dl). He is suffering from dyslipidemia, probably due to metabolic disorders caused by primary dyslipidemia, which was not treated effectively.

2. What is the biochemical basis behind the increase in serum total cholesterol and LDL-cholesterol?

Ans: Due to a deficiency of enzymes and proteins (apo-proteins) at his age, cholesterol metabolism, and transportation is affected.

3. Why he suffered from chest pain and abnormal changes in ECG?

Ans: High circulating serum cholesterol and LDL-cholesterol tend to deposit on coronary arteries leading to atherosclerosis and subsequent blockages. This patient might have suffered from myocardial infarction following strenuous physical activity.

4. What is the probable line of treatment?

Ans: Statins are prescribed to decrease total serum cholesterol and LDL cholesterol with controlled intake of lipids in diet, backed by regular exercises, as directed by the physiotherapist.

BAQ: Show horizontal integration of Case 4 with anatomy and physiology.

Ans: Horizontal integration with anatomy

Affected heart

Horizontal integration with physiology

Increased circulation of total cholesterol, and LDL-cholesterol

Horizontal integration with nutrition

The suggestion of a diet with a controlled intake of lipids and a diet free from high glycemic sugars like glucose, sucrose, maltose, and fructose (in table sugar, sweets, soft drinks, cakes, pastries, sweet fruits, etc.)

BAQ: Show vertical integration of symptoms and test reports of case 4 this patient with general medicine, pharmacology, and preventive medicine and pathology subjects

Ans: Vertical integration

General medicine: Study of effects of hypercholesterolemia on coronary arteries and heart functions

Pharmacology: Use of appropriate drug to treat primary hypercholesterolemia.

Preventive medicine: Preventive measures like diet low on lipids and high glycemic sugars. Appropriate exercises to control normal body mass index (BMI) to prevent insulin resistance.

Pathology: Study of causes of atherosclerosis, related heart diseases, diagnosis and prognosis.

Chapter
6
Proteins

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each
Case Studies: Marks: 4 or 5

INTRODUCTION

Proteins are high molecular-weight polymers of a group of low molecular-weight monomers called amino acids. These nitrogen compounds are the most abundant substances in cell protoplasm. The cell nucleus contains nucleoproteins, which are intimately associated with cell division and heredity. The cell cytoplasm contains a thousand or more separate proteins, in the form of enzymes, which catalyze the many chemical changes required for the maintenance of cell life. Animals, plants, and microbes produce extracellular enzymes which split complex dietary proteins, lipids, and carbohydrates into simple diffusible nutrients which are easily absorbed and utilized within the cell. Proteins also serve as major components of the blood, and various tissues, muscles, and many body fluids such as cerebrospinal fluid (CSF), semen, etc. Proteins also serve as a source of energy and fat.

AMINO ACIDS

Amino acids are the building blocks of proteins. The amino acids derived from animal, plant, or microbial proteins are all nitrogenous organic acids that contain either an amino or an amino group on the alpha carbon atom. A total of 18 amino acids have been isolated from the proteins of all major classes of living organisms. These are common to all living organisms. In addition, there are more than 80 amino acids, which have been isolated from the proteins or extracts of a single class of plant, animal, or microorganisms. The amino acids which cannot be synthesized from other substances by the body cells are called essential amino acids. Those amino acids which can be synthesized from other substances by the body cells are called nonessential amino acids. The essential amino acids are methionine, threonine, tryptophan, valine, isoleucine, phenylalanine, lysine, leucine, and histidine. The nonessential amino acids are glycine, alanine, serine, aspartic acid, glutamic acid, cystine, proline, hydroxyproline, etc.

SAQ: What is the importance of L-amino acids?

Ans: Twenty-two amino acids are naturally incorporated into polypeptides and are called standard amino acids. 20 amino acids from these are encoded by the universal genetic code. Eight standard amino acids are called "essential" for humans because they cannot be created from other compounds by the human body, and so must be taken in as food. Other standard amino acids are nonessential amino acids and can be synthesized in the body. L-amino acids occur in all proteins made by humans, animals, plants, bacteria, and fungi.

SAQ: What is the importance of D-amino acids?

Ans: Although natural proteins are formed by L-amino acids, D-amino acids are present in humans, plants, bacterial cell walls, and vertebrates. Significant quantities of D-amino acids have been found in dairy products such as cheese and yogurt, due to the fermentation process by microbial action.

SAQ: What is the difference between L-amino acids and D-amino acids?

Ans: The spatial arrangement of groups around the asymmetric carbon of amino acids is different as shown in the following structural formulae of L- and D-type of amino acid. When the "NH_2-group" is on the left-hand side of asymmetric carbon and "H" on the right-hand side, the amino acid is of "L" type. When "NH_2-group" is on the right-hand side of asymmetric carbon and "H" on the left-hand side, the amino acid is of "D" type (Fig. 6.1).

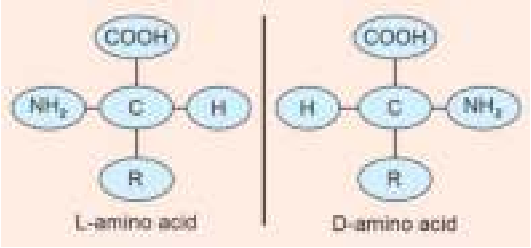


Fig. 6.1: Structural formulae of L- and D-amino acid

BAQ: Write a short note on D-amino acids.

Ans: D-amino acids are present in human brain tissue, teeth, eye lens, blood plasma, urine, saliva, cerebrospinal fluid (CSF), amniotic fluid, arterial walls, skin, and bones. D-amino acids in the body originate from the racemization of L-amino acids by racemase enzymes. The following two racemase enzymes have been found in mammals: Serine racemase and aspartate racemase. However, only serine racemase has been found in human tissue. Certain food processing techniques also contribute to the racemization of L-amino acids into D-amino acids in several types of food. Fermented foods such as vinegar and dairy products contain d-amino acids. According to recent evidence, the human gut microbiota may contribute to the systemic D-amino acids in the body.

D-serine (D-Ser) and D-aspartate (D-Asp) are the only D-amino acids in the human body originating from tissue intrinsic racemization. These two D-amino acids are involved in multiple processes in the central nervous system as well as in various endocrine tissues. D-Ser plays an important role in the activation of the n-methyl-d-aspartate (NMDA) receptor in the brain. D-Ser-based neurotransmission through the NMDA receptor is involved in learning and memory formation. Altered D-Ser signaling and metabolism have been found to contribute to NMDA receptor-linked neurophysiological disorders such as Alzheimer's disease (AD), epilepsy, and schizophrenia. D-Asp has also been found to be involved in NMDA receptor-linked neurotransmission and related diseases.

SAQ: What are essential amino acids?

Ans: Essential amino acids are not synthesized by body cells and it is necessary to derive these through appropriate food. The following are names of essential amino acids: Methionine, arginine, histidine, threonine, tyrosine, valine, isoleucine, leucine, and phenylalanine.

SAQ: What are glucogenic amino acids? Give examples.

Ans: Glucogenic amino acids are those that give rise to the formation of pyruvate or

TCA cycle intermediates, such as alpha-ketoglutarate or oxaloacetate, all of which are precursors to glucose via gluconeogenesis. All amino acids except leucine and lysine are at least partly glucogenic.

SAQ: What are ketogenic amino acids? Give examples.

Ans: Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetyl-CoA or acetoacetyl-CoA.

SAQ: What are glucogenic as well as ketogenic amino acids? Give examples.

Ans: Phenylalanine, threonine, isoleucine, tryptophan, and tyrosine give rise to both glucose and fatty acid precursors and are characterized as glucogenic and ketogenic.

Classification of Proteins

BAQ: Write a note on the classification of proteins with suitable examples.

Ans: Proteins can be classified as follows:

1. Fibrous proteins and
2. Globular proteins

Fibrous proteins are insoluble in all common solvents. Following are examples of fibrous proteins:

A. Keratin is the major constituent of epithelial tissues such as skin, hair, feathers, horn, and nails.
B. Collagen is the major protein constituent of white connective tissue of bone.
C. Elastin is the main protein constituent of yellow elastic fiber.
D. Fibroin is the major constituent of silk.

Globular proteins can be divided into:

1. Simple globular proteins and
2. Conjugated globular proteins.

Following are examples of simple globular proteins and these are soluble in water.

A. Albumin: Protein of serum, egg, and soybean.
B. Protamines: Protein of nucleoproteins and ripe sperm cells.
C. Histones: Protein of nucleoproteins.

Following are examples of simple globular proteins, which are not soluble in water, however, soluble in sodium chloride solution.

1. Globulin: Protein of serum, egg white, soybean, etc.

Conjugated proteins contain a specific non-protein component. Following are the examples of conjugated globular proteins:

A. Chromoproteins: Proteins combined with pigments. Example: Hemoglobin.
B. Glycoproteins: Proteins combined with carbohydrates. Example: Mucin (saliva)
C. Lipoproteins: Proteins combined with lipids. Example: Serum lipoproteins.
D. Nucleoproteins: Proteins combined with nucleic acid.
E. Phosphoproteins: Proteins combined with phosphoric acid. Example: Casein of milk.
F. Metalloprotein: Protein combined with metals. Example: Ceruloplasmin (copper-containing protein of serum).

STRUCTURE OF PROTEINS

Competency achievement: The student should be able to:

BI5.1: Describe and discuss the structural organization of proteins

LAQ: Write a note on the structural organization of protein.

Ans: Amino acids are the building blocks of proteins. The structure of the protein is formed of the following structural units:

1. Primary,
2. secondary,
3. Tertiary and
4. Quaternary.

Primary Structure of Proteins (Fig. 6.2)

Naturally occurring proteins contain 50–150,000 amino acid residues per molecule. Long chains of amino acids linked by the peptide bond are called polypeptides.

The number and order of the amino acids in the polypeptide chains are referred to as the “primary structure” of proteins. The simplest combination of amino acids that contains a peptide linkage is called a dipeptide. A molecule containing two peptide linkages (3 amino acids) is called a tripeptide, one containing three peptide linkages (four amino acids), a tetrapeptide and a molecule containing more than 10 peptides is called a polypeptide. Following is a simple example of a dipeptide:

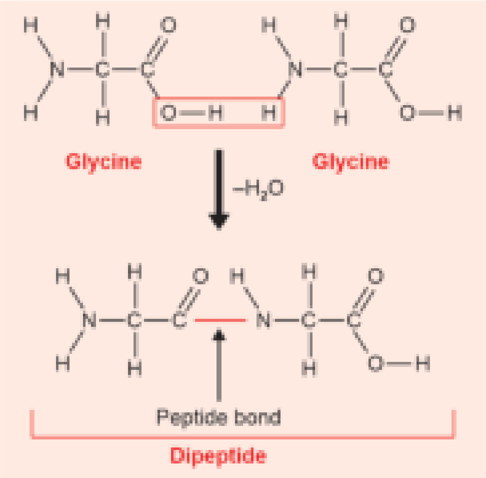


Fig. 6.2: Formation of the primary structure of the protein

Each peptide contains a free, terminal carboxyl group at the right-hand end of the chain and a free, terminal amino group at the left-hand end of the chain. A simple general formula for proteins would be as follows (Fig. 6.3):

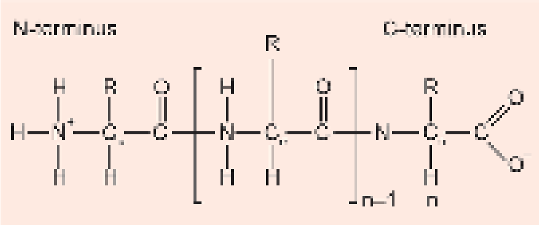


Fig. 6.3: General formula for protein

In the formula ‘R’ represents the distinctive aromatic or aliphatic side chains of any of the 18 amino acids.

Examples: The value for ‘n’ in the general formula is 51 for the hormone insulin, about 100 for simple proteins such as pepsin, and about 150,000 for complex nucleoproteins. The general formula for protein given above adequately portray their exceedingly complex structure.

The union of two peptide linkages can occur by S–S linkages and also by hydrogen bonds (Fig. 6.4).

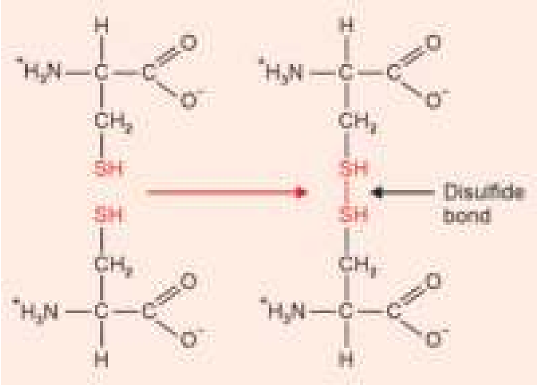


Fig. 6.4: Formation of disulfide linkage

NOTE

1. The primary structure refers to the amino acid sequence of the polypeptide chain.
2. The primary structure is held together by covalent or peptide bonds, which are made during the process of protein biosynthesis or translation.
3. The two ends of the polypeptide chain are referred to as the carboxyl terminus (C-terminus) and the amino terminus (N-terminus) based on the nature of the free group on each extremity. The counting of residues always starts at the N-terminal end (NH2-group), which is the end where the amino group is not involved in a peptide bond.
4. The folding of the polypeptide chains into a specific coiled structure held together by disulfide and hydrogen bonds is referred to as the secondary structure of proteins.

Secondary Structure of Proteins

This is concerned primarily with the arrangement of the polypeptide chain. The polypeptide backbone exists in different sections of protein in different forms such as alpha-helix, a beta-pleated sheet (or as a random coil).

The alpha-helix (Fig. 6.5)

In this, the polypeptide backbone is twisted into a right-hand helix. In the alpha-helix, the polypeptide chain is coiled tightly in the fashion of a spring. The "backbone" of the peptide forms the inner part of the coil while the side chains extend outward from the coil. The helix is stabilized by hydrogen bonds between the >N-H of one amino acid and the >C=O on the 4th amino acid away from it.

One "turn" of the coil requires 3.6 amino acid units. Each turn extends a distance of 0.54 nm, and the space between each amino acid is 0.15 nm. The naturally occurring alpha helixes found in proteins are all right-handed (Fig. 6.5).

NOTE

1. The right-handed alpha-helix is more stable than the left-handed.
2. All the peptide bonds except the first and last in the polypeptide chain participate in the formation of hydrogen bonds.
3. Amino acids such as proline disrupt alpha-helix, and some of the acidic amino acids (e.g. aspartic acid, glutamate), and basic amino acids (e.g. histidine, arginine and lysine, etc.), interfere with alpha-helix structure.

The beta-pleated sheet (Fig. 6.5)

The beta-pleated sheet is the second form of regular secondary structure in proteins. beta-sheets consist of beta-strands connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted, pleated sheet.

A beta-strand is a stretch of polypeptide chain typically 3 to 10 amino acids along with a backbone in a fully extended conformation.

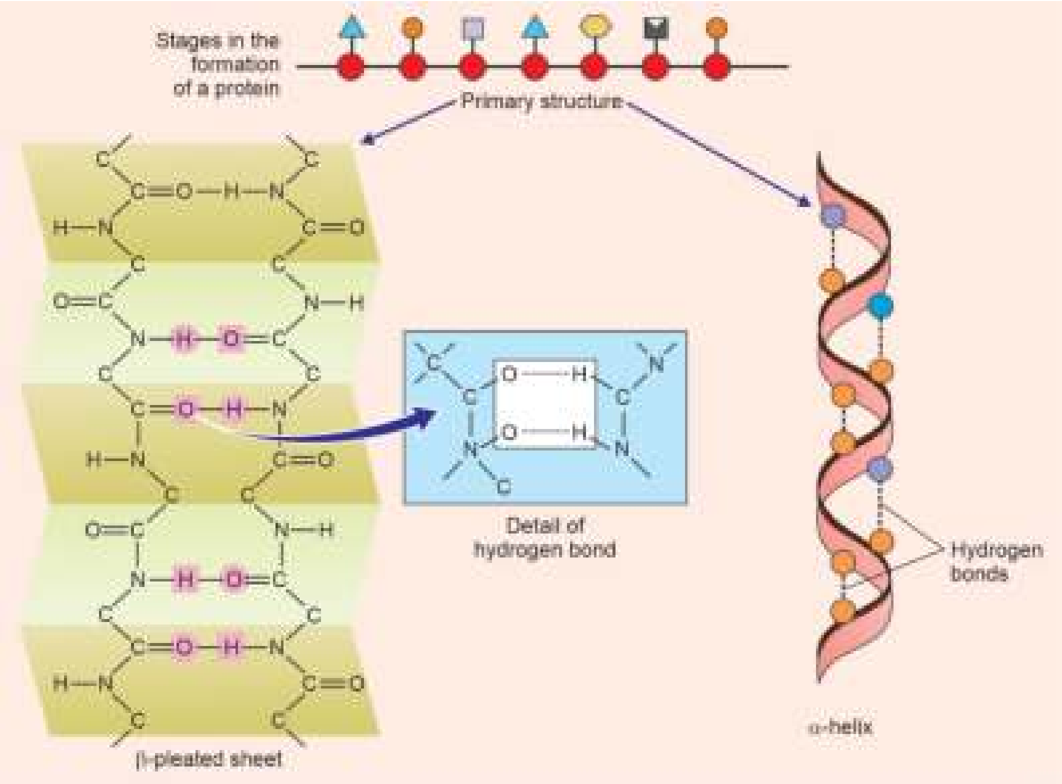


Fig. 6.5. α-helix and β-pleated sheet

The majority of β strands are arranged adjacent to other strands and form an extensive hydrogen bond network with their neighbor in which the N-H groups in the backbone of one strand establish hydrogen bonds with the C=O groups in the backbone of the adjacent strands.

In beta-sheets in a parallel arrangement, all of the N-termini of successive strands are oriented in the same direction. Large aromatic amino acids (e.g. tyrosine, phenylalanine, and tryptophan) and beta-branched amino acids (threonine, valine, and isoleucine) are favored to be found in beta-strands in the middle of beta-sheets. A beta-helix is formed from repeating structural units consisting of two or three short beta-strands linked by short loops. The side chains from the amino acid residues found in a beta-sheet structure may also be arranged such that many of the adjacent side chains on one side of the sheet are hydrophobic, while many of those adjacent to each other on the alternate side of the sheet are polar (hydrophilic), which can be useful if the sheet is to form a boundary between polar and nonpolar environments.

Tertiary Structure of Proteins (Fig. 6.6)

The secondary structures get folded and packed together to form the protein molecule. This arrangement of the various secondary structures into the compact structure of a globular protein is referred to as the tertiary structure (monomeric units of proteins). Tertiary structure refers to the three-dimensional structure of a single protein molecule. The alpha-helices and beta-sheets are folded into a compact globule. The folding is driven by the non-specific hydrophobic interactions. The structure is stable only when the parts of a protein domain are locked into place by specific tertiary interactions, such as salt bridges, hydrogen bonds, and the tight packing of side chains and disulfide bonds.

Quaternary Structure of Proteins (Fig. 6.7)

Many proteins (termed oligomers) possess many monomeric subunits held together by non-covalent bonds such as hydrogen and ionic bonds and hydrophobic interactions.

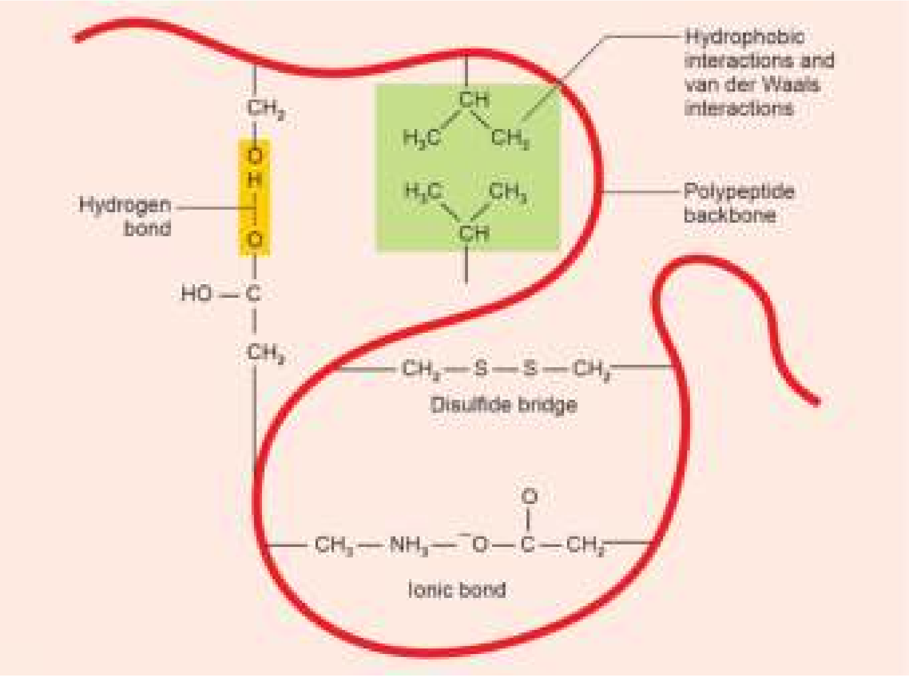


Fig. 6.6: Tertiary structure of a protein

A quaternary structure is a larger assembly of several protein molecules or polypeptide chains, usually called subunits. The quaternary structure is stabilized by the non-covalent interactions and disulfide bonds as the tertiary structure. Complexes of two or more polypeptides (i.e. multiple subunits) are called multimers. A quaternary structure would be called a dimer if it contains two subunits, a trimer if it contains three subunits, and a tetramer if it contains four subunits. The subunits are frequently related to one another by symmetry operations, such as a twofold axis in a dimer. Multimers made up of identical subunits are referred to as a homotetramer, and those made up of different subunits are referred to as a heterotetramer (e.g. the two alpha and two beta chains of hemoglobin) (Fig. 6.7).

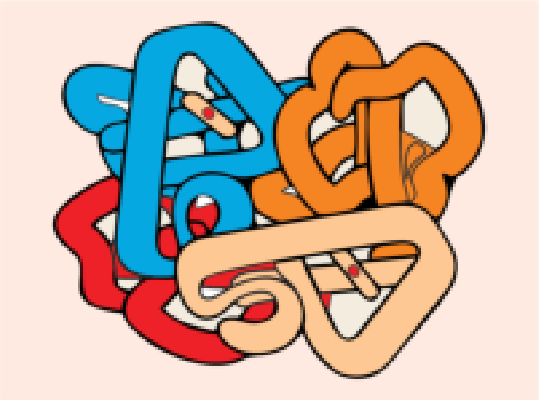


Fig. 6.7: Quaternary structure of a protein

Protein Folding

BAQ: Write a note on protein folding.

Ans: Protein folding is the physical process by which a polypeptide formed in protein synthesis folds into its characteristic and functional three-dimensional structure from the random coil, which emerges from the ribosomes. Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids. This polypeptide lacks any developed three-dimensional structure. Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence.

A protein molecule folds spontaneously during or after biosynthesis. This folding of protein process also depends on the following factors around: The type of solvent, present at the cellular level, salts concentration, the temperature, and the presence of specific proteins known as chaperones.

Folded proteins usually have a side chain packing which stabilizes the folded state, and charged or side chains occupy the solvent-exposed surface where they interact with the surrounding water. Minimizing the number of hydrophobic side chains exposed to water is an important driving force behind the folding process. The formation of intramolecular hydrogen bonds provides another important contribution to protein stability. The strength of hydrogen bonds depends on their environment; thus H-bonds enveloped in a hydrophobic core contribute more than H-bonds exposed to the aqueous environment to the stability of the native state.

The process of folding often begins co-translationally so that the N-terminus of the protein begins to fold while the C-terminal portion of the protein is still being synthesized by the ribosome.

Although most globular proteins can assume their native state unassisted, chaperone-assisted folding is often necessary. Chaperones are used to prevent misfolding and aggregation that may occur as a consequence of exposure to heat or other changes in the cellular environment.

Formation of quaternary structure usually involves the "assembly" or "co-assembly" of subunits that have already folded. The regular alpha-helix and beta sheet structures fold rapidly because they are stabilized by intramolecular hydrogen bonds.

Folding is a spontaneous process independent of energy inputs from nucleoside triphosphates. The passage of the folded state is mainly guided by hydrophobic interactions, the formation of intramolecular hydrogen bonds, and van der Waals forces.

SAQ: Enumerate external factors responsible for the folding of the protein.

Ans: Following external factors such as temperature, external fields (electric, magnetic), molecular crowding, and limitation of space have a big influence on the folding of proteins.

SAQ: What methods are used for the determination of the 3-D structure of a protein? What is protein sequencing? How protein sequencing is performed?

Ans: Three-dimensional structure of a protein is determined by X-ray crystallography or by NMR spectroscopy.

Protein sequencing means a determination of the order of amino acids in a protein molecule. Sequencing and amino acid composition of protein can be performed by mass spectroscopy or by Edman degradation using an automated protein sequenator.

Q: Name any two proteins with quaternary structure along with their subunits.

Ans:

1. Hemoglobin A (adult hemoglobin) with two subunits of an alpha chain and two subunits of a beta chain (Fig. 6.8).

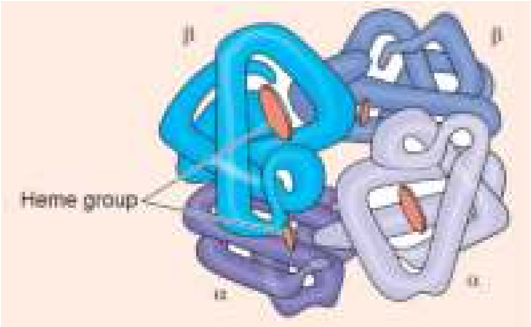


Fig. 6.8: Subunits of the quaternary structure of hemoglobin

2. Antibody IgG, made up of light and heavy chains (Fig. 6.9).

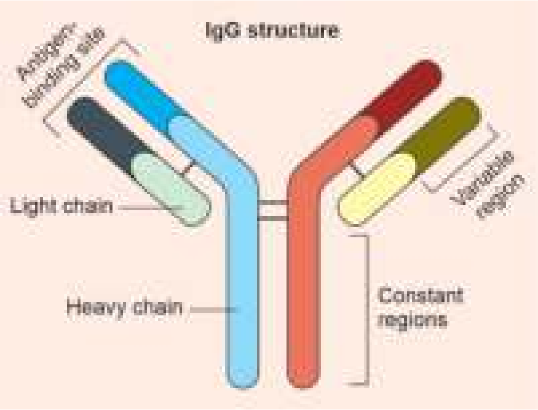


Fig. 6.9: Quaternary structure of antibody IgG

SAQ: What is misfolding of protein? What is the clinical significance of protein misfolding?

Ans: The correct three-dimensional structure of a protein is essential for protein functions. Exposure of cells to stress, particularly oxidative stress, leads to misfolding of proteins, and, if they are not refolded or degraded, cytoplasmic protein aggregates are formed.

SAQ: What is the clinical significance of protein aggregates? Mention two diseases related to protein aggregates.

Ans: Misfolding of protein leads to protein aggregates. Protein aggregates are characteristic features of a variety of chronic toxic and degenerative diseases, such as Mallory bodies (MBs) in hepatocytes in alcoholic and non-alcoholic steatohepatitis, neurofibrillary tangles in neurons in Alzheimer's disease, and Lewy bodies in Parkinson's disease.

BAQ: Write a note on protein misfolding and related diseases.

Ans: Exposure of cells to stress, particularly oxidative stress, leads to misfolding of proteins. Misfolding of protein leads to protein aggregates. Protein aggregates are characteristic features of a variety of chronic toxic and degenerative diseases, such as Mallory bodies (MBs) in hepatocytes in alcoholic and non-alcoholic steatohepatitis, neurofibrillary tangles in neurons in Alzheimer's, and Lewy bodies in Parkinson's disease.

Other diseases related to misfolded proteins are familial amyloid cardiomyopathy or polyneuropathy, and intracytoplasmic aggregation diseases such as Huntington's disease and Parkinson's disease.

SAQ: What are prions? Mention two diseases associated with prions.

Ans: Prions are misfolded proteins. Aggregated misfolded proteins form insoluble, extracellular aggregates and intracellular inclusions, including cross-beta sheet amyloid fibrils, which are associated with prion-related illnesses such as Creutzfeldt-Jakob disease and bovine spongiform encephalopathy (mad cow disease).

BAQ: Write a short note on chaperones.

Ans: Chaperones are proteins that assist the non-covalent folding or unfolding and the assembly or disassembly of other macromolecular structures. Chaperone, however, does not occur in finally folded proteins.

One major function of chaperones is to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into non-functional structures. Many chaperones are heat shock proteins. These are the proteins expressed in response to elevated temperatures or other cellular stresses. If protein folding is severely affected by heat, some chaperones act to repair the potential damage caused by misfolding.

Other chaperones are involved in folding newly made proteins as they are extruded from the ribosome. Some examples of chaperones are GRP94, GRP170 (general types), HSP47, and ERp29 (non-classical molecular type), protein disulfide isomerase (PDI, folding chaperone), etc.

Competency achievement: The student should be able to:

BI5.2: Describe and discuss functions of proteins and structure–function relationship in relevant areas, e.g. hemoglobin and selected hemoglobinopathies

IMPORTANT PROPERTIES OF PROTEINS

LAQ: Write a note on the properties of proteins.

Ans: The following are the important properties of proteins:

1. **Denaturation of proteins:** A protein is called a native protein if its amino acid composition and stereochemical structure are unchanged from the natural state. These properties control all the functions of the protein. These characteristics are altered when the protein is denatured. Denaturation is said to occur when a protein changes structure or composition. Chemical and physical agents which cause these changes are called denaturing agents. Denaturation results in an unfolding of the protein molecule due to the destruction of hydrogen bonds mainly. In general, denaturation consists of a series of changes in the protein molecule brought about by the various physical and chemical agents, and these changes often affect the viscosity, particle size, solubility, and even loss of certain amino acids or peptides of low molecular weight. Denatured proteins, because of their reduced solubility usually flocculate at or near the isoelectric pH (at which the protein is neutral).

   Denaturation may be caused by the various factors: Heat, mineral acids, mineral alkalies, alcohol, grinding, and vigorous shaking.
2. **Color reactions of proteins:** Biuret reaction: Peptide linkages of proteins and synthetic polypeptides react with biuret reagent (an alkaline 0.02% cupric sulfate solution containing Rochelle salt, i.e. Na, K-tartrate) to form a purple-colored complex. The

depth of the color formed (OD) is directly proportional to the concentration of protein present in the specimen. The reaction is given by those substances whose molecules contain at least two –CO–NH_2 groups and the end reaction is dependent upon the formation of a copper-potassium-biuret compound.

3. **Reaction with bromcresol green (BCG):** Albumin reacts specifically with bromocresol green at pH 4.1 to form a greenish-blue-colored complex. Since other proteins from the serum, such as globulin, do not react with BCG, this reaction gives a measure of serum albumin.
4. **Electrophoresis:** The movement of proteins in an electrical field is called electrophoresis. Proteins are amphoteric. They behave like colloidal particles in solution. Colloidal particles carry a charge and move when subjected to the electrical field. The rate of the migration of charged particles is dependent on the magnitude of charge carried by them. This depends on the molecular weight and size of the particles, their isoelectric pH, and also on the pH and composition of the surrounding medium. The isoelectric pH of a protein is the pH at which it is neutral and does not carry any charge. The isoelectric pH of serum albumin is 4.8, and that of globulin is 6.3. Other globulins have isoelectric pH values between 4.8 and 6.3. When a buffer such as a veronal buffer of pH 8.6 is used for electrophoresis, all the serum (or plasma) proteins behave like anions and move toward the anode. Since albumin has the smallest molecular size and weight, it carries a maximum charge, and hence it is the fastest-moving component. It is followed by alpha-1, alpha-2, beta-globulin, and gamma-globulin (Fig. 6.10). If plasma is subjected to electrophoresis, the fibrinogen band appears between beta-globulin and gamma-globulin. Electrophoresis is used to find out changes in the serum protein patterns in various diseases.

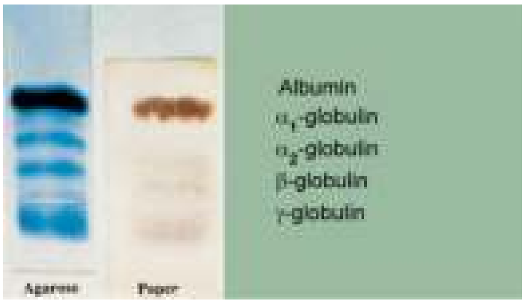


Fig. 6.10: Separated pattern of serum proteins by electrophoresis on paper and agarose

BAQ: What is the denaturation of proteins? Enumerate various factors that cause the denaturation of protein.

Ans: A protein is called a native protein if its amino acid composition and stereochemical structure are unchanged from the natural state. These properties control all the functions of the protein. These characteristics are altered when the protein is denatured. Denaturation is said to occur when a protein changes structure or composition. Chemical and physical agents which cause these changes are called denaturing agents. Denaturation results in an unfolding of the protein molecule due to the destruction of hydrogen bonds mainly. In general, denaturation consists of a series of changes in the protein molecule brought about by the various physical and chemical agents, and these changes often affect the viscosity, particle size, solubility, and even loss of certain amino acids or peptides of low molecular weight. Denatured proteins, because of their reduced solubility, usually flocculate at or near the isoelectric pH (at which the protein is neutral).

Denaturation may be caused by the following various factors:

a. Heat causes the splitting of the salt bridges by thermal agitation.
b. Mineral acids such as sulfosalicylic acid, tungstic acid, and hydrochloric and

sulfuric acid denature the protein by destroying the salt bridges by altering the ionization of carboxyl and amino groups.

c. Mineral alkalies such as sodium hydroxide and potassium hydroxide also denature the proteins by oxidative decomposition. Protein precipitation does not occur.
d. Shaking and stirring denature the proteins, leading to the unfolding of peptide chains.
e. Grinding causes the mechanical deformation of the peptide chain.
f. Alcohol and other organic solvents are protein precipitants.
g. Neutral chemical agents such as urea and guanidine derivatives cause cleavage of hydrogen bonds.
h. Ultraviolet radiation splits the peptide bonds adjacent to the aromatic rings.
i. Ultrasonic waves destroy the ring of aromatic amino acids, depending upon their frequency.

BAQ: What is electrophoresis?

Ans: The movement of colloidal particles like proteins in an electrical field is called electrophoresis. Proteins are amphoteric. They behave like colloidal particles in solution. Colloidal particles carry a charge and move when subjected to the electrical field. The rate of the migration of charged particles is dependent on the magnitude of charge carried by them. This depends on the molecular weight and size of the particles, their isoelectric pH, and also on the pH and composition of the surrounding medium. The isoelectric pH of a protein is the pH at which it is neutral and does not carry any charge. The isoelectric pH of serum albumin is 4.8, and that of globulin is 6.3. Other globulins have isoelectric pH values between 4.8 and 6.3. When a buffer such as a veronal buffer of pH 8.6 is used for electrophoresis, all the serum (or plasma) proteins behave like anions and move toward the anode. Since albumin has the smallest molecular size and weight, it carries a maximum charge, and hence it is the fastest-moving component. It is followed by alpha-1, alpha-2, beta-globulin, and gamma-globulin (Fig. 6.10).

Electrophoresis is used to find out changes in the serum protein patterns in various diseases. In severe liver disease, albumin is decreased and gamma-globulin is increased. Similarly, in severe kidney disease, albumin is decreased and beta-globulin is increased. In multiple myeloma, an additional "M" band appears with serum proteins, and in cirrhosis of the liver, a decrease in albumin is observed (Fig. 6.11).

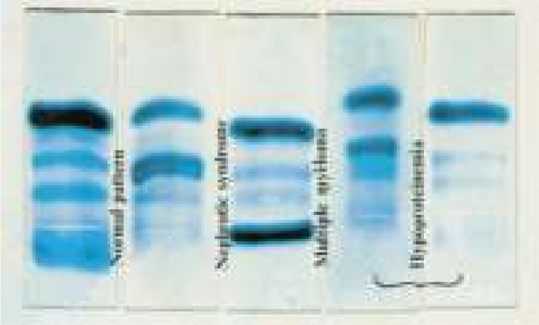


Fig. 6.11: Changed serum protein pattern in various diseases

STRUCTURE–FUNCTION RELATIONSHIP OF PROTEINS

LAQ: Write a note on the structure and function relationship of proteins.

Ans: The proteins can exhibit a variety of functions due to their characteristic specific structures. The three-dimensional structural conformation, which is dependent on the primary structure of the protein, provides and maintains these functional characteristics. The side chains of the amino acid residues are involved in the formation of hydrogen bonds, electrostatic bonds, and van der Waals' forces to bind with other molecules. The following different types of proteins express very well the structure–function relationship:

Transport Protein

Hemoglobin is a tetrameric protein, with each monomer having a heme unit (Fig. 6.8). The binding of oxygen to one heme facilitates oxygen binding by other subunits. The binding of H^+ and CO_2 promotes the release of O_2 from hemoglobin. These allosteric interactions are physiologically important (termed Bohr effect). However, in the primary structure, even a single amino acid substitution alters the normal specific structure of hemoglobin. In sickle cell anemia, the 6th amino acid in the beta chain is altered, leading to profound clinical manifestations such as the decrease in life span of red blood cells that cause "severe hemolytic anemia" (Fig. 6.12).

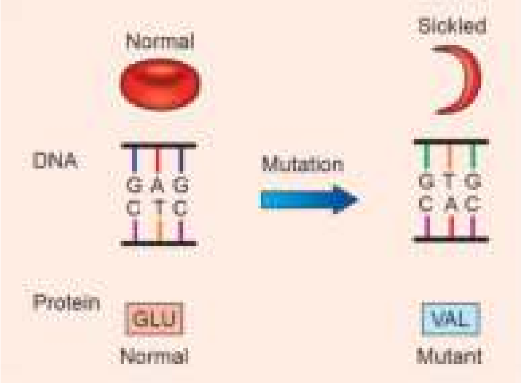


Fig. 6.12: Genetic primary structure defect of protein leading to sickle cell anemia

Contractile Protein

Myosin is a large protein present in muscles. It has 6 polypeptide chains, two identical heavy (H) chains, and four light (L) chains. The functional fragments of myosin are called light meromyosin (LMM) and heavy meromyosin (HMM). The LMM can form filaments, does not combine with actin, and has no ATPase activity. HMM has ATPase activity and binds actin, but does not form filaments. Myosin ADP–Pi complex binds actin and releases Pi and ADP by causing a major conformational change in the molecule leading to the movement of the filament, and muscle contraction.

Structural Protein

The main structural component of skin, bone, teeth, cartilage, and tendon is collagen, a fibrous protein. Collagen represents a super helical cable where the three polypeptide chains are wound around each other. In this structure, nearly every 3rd residue is a glycine, which is the only amino acid that can fit into the triple-stranded helix. This triple helix of collagen is stabilized by the steric repulsion of the rings of proline and also by the hydrogen bonds between them. In vitamin C deficiency, failure of hydroxylation of proline/lysine leads to reduced hydrogen bonding and consequent weakness of collagen. The typical triple helical structure of collagen is responsible for its tensile strength.

SAQ: Enumerate four hematological disorders caused due to genetic defects in the globin structure of hemoglobin.

Ans: Sickle cell trait, Sickle cell anemia, alpha-thalassemia, beta-thalassemia.

BAQ: Write a short note on plasma proteins

Ans: The concentration of total protein in human plasma is approximately 7.0–7.5 g/dl and comprises the major part of the solids of the plasma. The plasma proteins are a complex mixture of major simple proteins such as albumin, various globulins such as alpha-1, alpha-2, beta-globulin and gamma-globulin, and fibrinogen (Fig. 6.13). Other types of proteins present in plasma are glycoproteins, lipoproteins, complement, enzymes, and various coagulation factors.

Most plasma proteins, except for immunoglobulins and protein hormones, are synthesized in the liver. They are secreted by the hepatocytes into the space of Disse and then move into the bloodstream through the hepatic sinusoids, which open into the central veins of the liver. Gamma-globulins originate from plasma cells and lymphoid tissue. The reticuloendothelial system participates in the formation of antibodies.

BAQ: Write a short note on prealbumin and retinal-binding protein (RBP).

Ans: Pre-albumin and retinal-binding proteins are synthesized in the liver. The

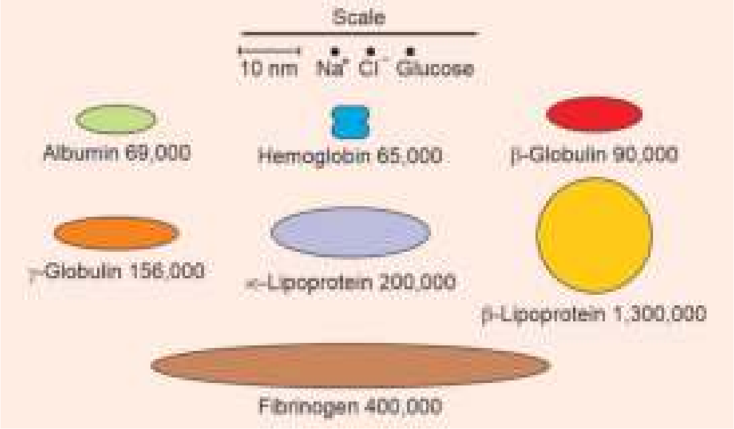


Fig. 6.13. Major serum proteins

molecular weight of albumin is 54,000 and that of RBP is 21,000. Both are transport proteins. Prealbumin binds thyroxin (T_4) and triiodothyronine (T_3). RBP transports vitamin A (retinol).

Serum prealbumin level falls in inflammation, cirrhosis of the liver, malignancy, and protein-wasting diseases of the gut or kidneys. In Hodgkin's disease, the prealbumin level rises. Decreases in RBP are associated with liver disease, protein malnutrition, and zinc deficiency states. Serum RBP increases in chronic renal disease.

During electrophoresis, both prealbumin and RBP migrate ahead of albumin. Their half-lives are about 12 hours. The reference ranges of prealbumin and RBP are 10–40 mg/dl and 3.5–9.0 mg/dl, respectively, in adults. Serum prealbumin and RBP determinations are performed by methods based on nephelometry and turbidimetry.

BAQ: Write a short note on albumin.

Ans: The name albumin is derived from the formation of white-colored (Latin: *Albus* means white) precipitate when acidic urine (containing albumin) is boiled. It is the most abundant protein in human plasma, representing 40–60% of the total proteins. It is synthesized in the liver, and the rate of synthesis is dependent on protein intake but subject to feedback regulation by the plasma albumin level. Albumin does not contain carbohydrates, and it is not stored to any extent in parenchyma cells. The half-life of albumin has been estimated at 15 to 19 days. Normally, traces of albumin can be found in almost all extravascular body fluids, and a little is lost from the body by excretion. The small concentration of albumin in the glomerular filtrate (0.04%) is probably reabsorbed by the proximal tubular cells and degraded by their lysosomal enzymes into fragments, which then return to circulation. Albumin is catabolized in various tissues, where it is taken up by pinocytosis. Its constituent amino acids are released by intracellular proteolysis. These amino acids then return to the body pool.

The molecular weight of albumin is approximately 66,000. The isoelectric pH (pI) of albumin is between 4 and 5.8 (average 4.8). At the normal pH of blood (about 7.4) it acts as an anion with more than 200 negative charges per molecule. The most important biological functions of albumin are to transport and store a wide variety of ligands, to maintain the plasma oncolytic plasma pressure, and to serve as a source of endogenous amino acids. Due to the large number of charges on each molecule, albumin can bind nonpolar compounds such as bilirubin and long-chain fatty acids. It also binds to thyroxin (T4), triiodothyronine (T3), cortisol, and aldosterone. About 40% of serum calcium is bound to albumin. Drugs such as phenylbutazone, warfarin, clofibrate, and salicylate also bind to albumin.

Serum albumin levels increase in dehydration (hyperalbuminemia). Decreased values of serum albumin (hypoalbuminemia) are observed in most instances from one or more of the following factors: In malnutrition, loss from urine (as in renal disease), and decreased synthesis (as in severe liver disease).

BAQ: What is hypoalbuminemia and prominent symptom of hypoalbuminemia? Enumerate causes of hypoalbuminemia.

Ans: Normal range of serum albumin is 3.8–4.8 g/dl. Hypoalbuminemia means a significant decrease of protein in serum, i.e. below 3.8 g/dl. In this clinical condition, the patient suffers from edema mainly of the face and feet, due to the flow of water from the blood to tissue spaces. Since, water flows from high water potential (blood, due to low proteins) compartment to low water potential (tissue spaces). Various reasons for a decrease in plasma albumin are:

1. Impaired synthesis, either primary as in liver disease or secondary due to diminished protein intake.
2. Increased catabolism as a result of tissue damage and inflammation.
3. Reduced absorption of amino acids caused by malabsorption syndromes or malnutrition.
4. Loss of protein:
    - In urine, due to nephrotic syndrome, chronic glomerulonephritis, diabetes, or systemic lupus erythematosus.
    - In feces, due to protein -losing enteropathy arising from the inflammatory or neoplastic disease.
    - From skin, through burns.
    - In altered distribution, as in ascites, circulation drives albumin in peritoneal fluid.

BAQ: Describe various functions of serum proteins.

Ans: The following are the various functions of serum proteins:

1. Water exchange: An important function of the serum proteins is the maintenance of osmotic relations between the circulating blood and the tissue spaces. Decreases in serum proteins or increases in venous pressure (as in heart disease) are examples of pathologic processes which, by altering the balance between osmotic and hydrostatic pressure, would lead to edema. Albumin is a major serum protein, constitutes about 55 to 60% of total proteins, and plays a very important role in maintaining serum osmotic pressure.
2. Blood buffers: The serum proteins, like other proteins, are amphoteric and can combine with acids and bases. At the normal pH of the blood, the proteins act as an acid and combine with cations (mainly sodium).
3. A reserve of body protein: The circulating plasma protein is not static. It constantly interchanges with a labile tissue reserve equal in quantity to the circulating protein. The term "dynamic equilibrium" has been applied to this interchange. In protein starvation, the body draws upon this tissue reserve as well as upon plasma proteins for its metabolic needs.
4. Transport of various substances: Serum proteins carry out the transport of various substances such as lipids, fat-soluble vitamins, steroids, hormones, antibodies, and various carbohydrates.

Albumin can bind various ligands. These include free fatty acids (FFA) and tryptophan. It also binds to copper and a variety of drugs such as sulfonamides, penicillin G, dicumarol, and aspirin.

SAQ: What is haptoglobin? Write an important function of haptoglobin.

Ans: Haptoglobin (Hp): It is a plasma glycoprotein that binds extra-corpuscular hemoglobin in a tight non-covalent complex (Hp–Hb). Approximately 10% of the hemoglobin that is degraded each day is released into the circulation and is extracorpuscular. Free hemoglobin passes through the glomerulus of the kidney, enters the tubules, and tends to precipitate therein. However, the Hp–Hb complex is too large to pass through the glomerulus. The function of Hp thus appears to prevent the loss of free hemoglobin in urine. This conserves the valuable iron present in hemoglobin, which would otherwise be lost to the body.

BAQ: What is transferrin? Write the important function of transferrin and the clinical significance of the serum transferrin test.

Ans: Transferrin is a beta-1-globulin, a glycoprotein synthesized in the liver. It acts as a transport vehicle for iron. Transferrin transports iron (2 mols of Fe^{++} per mole of transferrin) in the circulation to sites where iron is required, e.g. from the gut to the bone marrow and other organs. Iron is required for the synthesis of hemoglobin, myoglobin, and cytochromes.

Approximately 200 billion red blood cells (about 20 ml) are catabolized every day, that release about 25 mg of iron into the body. Free iron is toxic, however, its combination with transferrin diminishes its potential toxicity. Transferrin then directs iron to where it is required in the body. Many cells have receptors on their surfaces. The protein binds to these receptors, and then it is internalized by receptor-mediated endocytosis.

The concentration of transferrin in plasma is approximately 300 mg/dl. This amount of transferrin can bind 300 µg of iron/dl so this represents the total iron-binding capacity of plasma. However, the protein is normally only one-third saturated with iron. In iron deficiency anemia, the protein is even less saturated with iron. In hemochromatosis, when iron is deposited in various tissues, the saturation of transferrin with iron is much greater than one-third.

BAQ: What is ferritin? Write the important function of ferritin and the clinical significance of the serum ferritin test.

Ans: Ferritin is a protein that stores iron which can be used when the iron is required for a specific purpose like a synthesis of hemoglobin. Ferritin contains approximately 23% iron. Normally the concentration of ferritin in plasma is quite low. Hemochromatosis is a clinical condition in which excess iron is deposited in various organs of the body. In this clinical condition, body stores of iron are greatly increased, and high levels of ferritin are present in liver and spleen tissues. Hemosiderin is a partly degraded form of ferritin, and it also contains iron. It can be detected by histologic stains. The presence of hemosiderin is determined histologically when excessive storage of iron occurs.

BAQ: What is ceruloplasmin? Write the important function of ceruloplasmin and the clinical significance of the serum ceruloplasmin test.

Ans: Ceruloplasmin: It is an alpha-2-globulin. It carries 90% copper present in plasma. It has blue color due to its high copper content. Each molecule of ceruloplasmin binds six atoms of copper very tightly. Albumin carries the other 10% of plasma copper. Ceruloplasmin exhibits a copper-dependent oxidase activity. The amount of ceruloplasmin in plasma is decreased in liver disease. Low levels of ceruloplasmin are found in Wilson's disease, which is caused due to abnormal metabolism of copper.

SAQ: What is Wilson’s disease?

Ans: Wilson's disease is a genetic disease in which copper is not able to get circulated due to a congenital deficiency of a specific protein, ceruloplasmin, that transports copper. Copper fails to get excreted in the bile and accumulates in the liver, brain, kidney, and red blood cells, resulting in copper toxicosis. As the amount of copper accumulates in various tissues, patients may develop hemolytic anemia, chronic liver disease, and neurological syndrome.

SAQ: Enumerate four prominent features of Wilson's disease.

Ans: Enlarged liver, pallor, jaundice, hemolytic anemia.

BAQ: What is C-reactive protein (CRP)? Write the clinical significance of the serum CRP test.

Ans: C-reactive protein is synthesized in the liver. It consists of five identical non-glycosylated polypeptide subunits noncovalently linked to form a disc-shaped cyclic polymer. The molecular weight of CRP is 114000–140000. CRP is important in the nonspecific host defense against inflammation caused mainly by infection.

CRP concentration normally present in serum is at a mean concentration of less than 800 µg/dl. CRP levels in plasma rise after myocardial infarction, infection, inflammation, surgery, stress, trauma, or in neoplastic proliferation. The increase occurs within 24 to 48 hours, and the level may be 2000 times higher than the normal levels. The increase in inflammation occurs within 6–12 hours and peaks at the end of two days (48 hours).

CRP mainly binds to polysaccharides present in many bacteria, fungi, and protozoal parasites. Once complexed, CRP becomes an activator of the classical complement pathway. Like antibodies, CRP can initiate opsonization, phagocytosis, and lysis of invading cells, as a response to the inflammatory reaction.

SAQ: What are acute phase proteins (APP)? Write the clinical significance of an increase in APP.

Ans: Fibrinogen, ceruloplasmin, C-reactive protein, haptoglobin, and alpha-1-antitrypsin, etc. are acute phase proteins (APP) and their levels increase 50 to 1000 folds in various inflammatory and neoplastic conditions.

MECHANISM OF PHYSIOLOGIC HEMOSTASIS (Fig. 6.14)

LAQ: What are coagulation factors? Describe their roles in hemostasis.

Ans: About twelve main coagulation factors are present in circulating plasma and the tissue surrounding the blood vessels. These factors are protein in nature and are in the inactive (zymogen) form under normal circulating conditions. Whenever bleeding takes place, they are activated, and by their action, blood coagulation takes place. Following is the list of the coagulation factors:

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin
IV	Calcium ions
V	Labile factor (proaccelerin)
VI	Accelerin exists in combined with factor 'V' (Va)
VII	Stable factors (proconvertin)
VIII	Antihemophilic factors 'A' (AHF)
IX	Plasma thromboplastin component (PTC) (or antihemophilic factor 'B' or Christmas factor)
X	Stuart-Prower factor
XI	Plasma, thromboplastin antecedent
XII	Hageman (or contact) factor
XIII	Fibrin stabilizing factor.

Other required components are the following two proteins: Prekallikrein and high molecular weight kininogen (HMWK)

Platelets, endothelial cells, neutrophils, and monocytes take part in the first part of the mechanism of physiologic hemostasis. In the second part of physiologic hemostasis, a large number of plasma proteins play important functions, which include clot formation (coagulation), dissolution of the clot (fibrinolysis), and termination of activity of several enzymes of the coagulation and

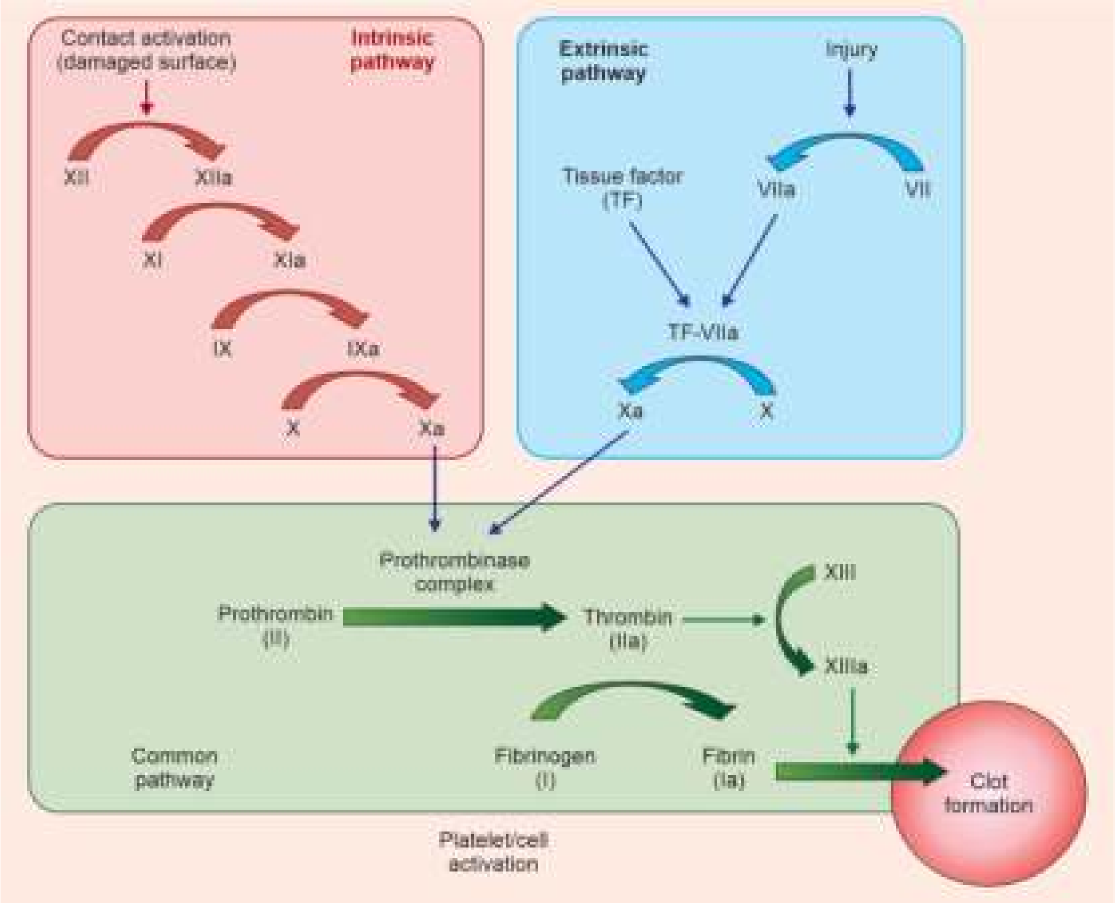

Contact activation
(damaged surface)
Intrinsic pathway
XII
XIIa
XI
XIa
IX
IXa
X
Xa
Extrinsic pathway
Injury
Tissue factor
(TF)
VIIa
VII
TF-VIIa
Xa
X
Prothrombinase complex
Prothrombin
(II)
Thrombin
(IIa)
XIII
XIIIa
Common pathway
Fibrinogen
(I)
Fibrin
(Ia)
Clot formation
Platelet/cell activation

Fig. 6.14: Mechanism of physiologic hemostasis

fibrinolytic systems (performed by naturally occurring serine protease inhibitors).

The first part of mechanism of physiologic hemostasis (roles of platelets and endothelium)

Collagen is exposed when a vessel wall is injured. Platelets adhere to the site of injury. von Willebrand factor helps platelets to adhere to the exposed collagen and the platelet receptor GP Ib-IX-V complex. Due to platelet adhesion to the site of injury, they get activated, and a signaling cascade initiates. The stimulated platelets release contents of their granules, which generate thrombin formation on their surface, leading to platelet aggregate and a hemostatic plug formation.

Adjacent to the site of injury, tissue factor (TF) is upregulated in the subendothelium. Tissue factor (TF) is a membrane-bound lipoprotein and is constitutionally present in many tissues outside the vasculature and on the surface of stimulated inflammatory cells such as monocytes. Upregulation of tissue factor leads to the formation of a complex with factor VIIa and the following reactions:

- The complex TF-VIIa activates factor IX to factor IXa.
- Zymogen factor X is converted to enzymatically active Xa by the action of factor IXa.
- In the presence of factor Va, factor Xa activates prothrombin (factor II) to thrombin (factor IIa). Thrombin is the major clotting enzyme.
- Thrombin then acts on soluble fibrinogen to form insoluble fibrin.

The second part of the mechanism of physiologic hemostasis (roles of coagulation protein system)

A damaged blood vessel creates a negatively charged surface leading to the activation of factor XII. The autoactivation of factor XII leads to activating prekallikrein and further amplification of factor XII. Amplified factor XII activates XI with the formation of factor Xa, which induces a series reactions, leading to coagulation process. Proteolytic reactions: The second part of the mechanism of physiologic hemostasis takes place as follows:

- Prekallikrein, when assembled on endothelial cells, is first activated by an endothelial enzyme, which activates factor XII.
- Factor XII is activated to factor XIIa by the proteolytic action of kallikrein.
- Factor XIIa, once formed, activates factor XI to XIa.
- Factor XIa in the presence of Ca^{2+} activates factors IX to factor IXa.
- Factor VIII (antihemophilic factor), when activated to factor VIIIa by thrombin, acts as a cofactor for factor IXa and also activates factor X to factor Xa.
- In common pathways, factor Xa acts as a cofactor in the conversion of prothrombin to thrombin, which acts on fibrinogen to form fibrin (Fig. 6.14).

The deposition of fibrin and its removal is regulated by the fibrinolytic system. This is a complex multicomponent system with many activators and inhibitors. Plasmin forms from plasminogen (circulating inactive precursor) and acts on fibrin, and leads to its dissolution.

BAQ: What are hemorrhagic disorders?

Ans: Clinically, hemorrhagic disorders are characterized by

1. Spontaneous bleeding into internal tissues, mucous membranes, and skin
2. Excessive bleeding following trauma and surgery, and
3. Bleeding from more than one site.

A breakdown in the normal hemostatic mechanism may result from a defect in any one of the following components: Vascular, platelet, and coagulation components. Bleeding may take place when more than one component is defective.

Hemorrhagic disorders due to vascular defects (non-thrombocytopenic purpura)

Vascular defects are a common cause of bleeding disorders seen in clinical practice. Most cases of bleeding due to a vascular defect are not severe. Frequently, the bleeding is mainly into the skin, causing petechiae or ecchymoses, or both. Vascular defects may be acquired or congenital.

- Acquired
  Infections (typhoid fever, gram-negative septicemia, smallpox, subacute bacterial endocarditis)
  Drugs (penicillin, aspirin, barbiturates, antihistamines, etc.)
  Scurvy
  Cushing's syndrome
  Mechanical purpura
- Congenital
  Hereditary hemorrhagic telangiectasis
  Ehlers-Danlos disease

Hemorrhagic disorders due to thrombocytopenia (decrease in platelets):

These are caused due to a significant reduction in the peripheral blood platelets. Following are the various reasons that lead to thrombocytopenia:

Drugs and chemicals
Aplastic anemia
Leukemia
Hypersplenism
Severe liver disease
Alcoholism
Massive blood transfusion
Heparin therapy
Vitamin K deficiency

Hemorrhagic disorders due to deficiency of coagulation factors:

1. Hemophilia A: Hemophilia is a sex-linked disorder and, accordingly, with rare exceptions, afflicts the males. Identical

clinical disease results from a deficiency of factor VIII in about 85% of cases (hemophilia A) or of factor IX in 15% of cases (hemophilia B).

2. von Willebrand's disease (vWD): A central role of vWF is it plays an important role in the adhesion of platelets to subendothelial surfaces following vessel injury.

vWD appears to comprise a heterogeneous group of disorders, in which qualitative and/or quantitative abnormalities of vWF are present due to genetic disorders.

SAQ: Name two diseases related to deficiency of coagulation factors.

Ans:

1. Hemophilia
2. von Willebrand's disease (vWD)

SAQ: Name two hemorrhagic disorders due to vascular defects.

Ans:

1. Scurvy
2. Bacterial endocarditis

SAQ: Name the disease and one therapy related to thrombocytopenia.

Ans:

1. Aplastic anemia
2. Heparin therapy

PROTEIN DIGESTION

Competency achievement: The student should be able to:

BI5.3: Describe digestion and absorption of dietary proteins

LAQ: Write a note on protein digestion and absorption.

Ans: Proteins form a very important constituent of the diet. They are mainly involved in building body tissues and supplying energy. The main sources of protein are milk, egg, meat, liver, fish, nuts, pulses, beans, and cereal grains.

Mastication subdivides the food, increasing its solubility and surface area for enzyme attack. Protein digestion, however, begins in the stomach. Proteins are denatured by hydrochloric acid secreted by the parietal cells of the stomach. Pepsinogen (inactive) secreted by the chief cells of the stomach is activated to pepsin by hydrochloric acid. Pepsin acts on denatured proteins (pH 1–2), and the resultant products are large polypeptide derivatives, peptones, and proteoses.

Rennin is one enzyme secreted by the cells of the stomach of infants. Rennin in present as inactive form as prorenin and it is converted into active form to rennin by hydrochloric acid. The enzyme acts on the casein of milk and is responsible for the curdling of the milk.

Stomach contents (chyme) are intermittently introduced into the duodenum, and digestion continues in the small intestine.

The duodenum can regulate its environment with hormones that are released from the duodenal epithelium. These hormones are secretin and cholecystokinin. Secretin is released when the pH of the duodenum decreases towards an alkaline level. This hormone acts to neutralize the pH of the duodenum by stimulating water and bicarbonate secretion into the duodenum.

The pancreatic juice secreted in the small intestine during digestion contains enzymes—trypsin, chymotrypsin, and carboxypeptidase in zymogen forms. Trypsinogen is converted to active form trypsin by enterokinase enzyme secreted by the duodenum wall. Trypsin acts as follows:

$$\text{Protein, proteoses} \xrightarrow[\text{pH 5.2–6}]{\text{Trypsin}} \text{Polypeptides, dipeptides}$$

$$\text{Protein, proteoses, peptones} \xrightarrow{\text{Chymotrypsin}} \text{Polypeptides, dipeptides}$$

The following enzymes present in the brush border of the small intestine complete the digestion of remaining polypeptides, and dipeptides and end products of protein digestion are free amino acids: Carboxypeptidase, aminopeptidase, and dipeptidase.

Polypeptides with free carboxyl group $\xrightarrow{\text{Carboxypeptidase}}$ Lower peptides, free amino acids

Following secretions of the glands of the duodenum also act as follows:

Polypeptides with free amino groups $\xrightarrow{\text{Aminopeptidase}}$ Lower peptides, free amino acids

Dipeptides $\xrightarrow{\text{Dipeptidase}}$ Amino acids

The end products of protein digestion are absorbed as individual amino acids, into portal blood. These amino acids are actively transported across the intestine from the mucosa to the serosa. Vitamin B_6 is involved in this transfer. Absorption of amino acids takes place by energy-dependent active transport.

ABSORPTION OF AMINO ACIDS

Under normal circumstances, the dietary proteins are almost completely digested into their constituent amino acids. These are then rapidly absorbed from the intestine into the portal blood. L-amino acids are absorbed at a faster rate compared to D-amino acids. Free amino acids are then taken into the enterocytes by a sodium-linked secondary transport system. Di- and tripeptides are taken up by a hydrogen-linked transport system. The peptides are broken there in the cytosol to amino acids and then released into the portal system.

Mechanism of Amino Acid Absorption

Like D-glucose, amino acids are also absorbed by sodium-dependent active transport. Sodium ions diffuse with a density gradient and with these ions, amino acids enter intestinal cells with a common carrier. Energy to this amino acid absorption mechanism is provided by ATP molecules. The meister cycle or gamma-glutamyl cycle is responsible for the transportation of cysteine and glutamine in the intestine, brain, and kidney tubules. Glutathione plays an important role in this transport mechanism.

SAQ: Write a note on allergic reactions that take place due to specific degraded products of protein molecules.

Ans: Dipeptides and tripeptides that escape digestion in the small intestine, can enter the brush border of mucosal cells of the small intestine. These are then hydrolyzed in amino acids, which enter general blood circulation through the portal vein. These amino acids may be responsible for allergic reactions that cause nausea, abdominal pain, diarrhea, or vomiting.

SAQ: Why some individuals are advised to avoid specific foods that contain wheat?

Ans: Some individuals are allergic to gluten present in wheat, which may cause allergic reactions such as nausea, vomiting, indigestion, stomach cramps, diarrhea, skin rash, etc. Children may suffer from growth retardation and weight loss, if this problem remains undiagnosed.

SAQ: What is gluten allergy? How gluten allergy is diagnosed?

Ans: Some individuals are allergic to gluten present in wheat, which may cause allergic reactions such as nausea, vomiting, indigestion, stomach cramps, diarrhea, skin rash, etc. Gluten allergy is diagnosed by detecting gluten antibodies in the serum.

SAQ: Enumerate names of allergy-causing foods for some individuals. What precautions are advised for such patients?

Ans: Eggs, cow's milk, wheat, shellfish, soyabean, etc. These individuals can safely avoid eating allergy-causing foods.

AMINO ACID METABOLISM

LAQ: Write a note on amino acid metabolism.

Ans: Digestion of proteins from a normal diet results in a relatively large amount of 20 different types of amino acids being absorbed from the intestine into portal blood, which immediately pass to the liver and then to other cells of the body. The liver is the major site of nitrogen metabolism in the body. During the fed state (anabolic phase), amino acids are used for the synthesis of various tissue and blood proteins. During the fasting phase (catabolic phase), amino acids are metabolized in various pathways such as the urea cycle (formation of urea), transamination (formation of new nonessential amino acids), decarboxylation (formation of amine like dopamine), oxidative deamination (formation of keto acids), etc.

All tissues have the capability for the synthesis of non-essential amino acids. The tissues are also capable of amino acid remodeling, and conversion of non-amino acid carbon skeletons into amino acids and other derivatives that contain nitrogen. Amino acids fall into three categories: Glucogenic, ketogenic, or glucogenic as well as ketogenic.

Glucogenic amino acids are those that give rise to the formation of pyruvate or TCA cycle intermediates, such as alpha-ketoglutarate or oxaloacetate, all of which are precursors to glucose via gluconeogenesis. All amino acids except leucine and lysine are at least partly glucogenic.

Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetyl-CoA or acetoacetyl-CoA.

Phenylalanine, threonine, isoleucine, tryptophan, and tyrosine give rise to both glucose and fatty acid precursors and are characterized as glucogenic and ketogenic.

Amino acids have a third possible fate. During times of starvation, the reduced carbon skeleton can be used for energy production, and the end products are CO_2 and H_2O.

All cells, except erythrocytes, use amino acids for protein synthesis and also for the synthesis of a variety of substances such as enzymes, protein hormones, membrane components, neurotransmitters, heme, plasma proteins, etc.

Each day, humans turn over 1–2% of their total body protein, principally muscle protein. The liberated 75–80% of amino acids are reutilized for new protein synthesis. The nitrogen of 20–25% of amino acids forms urea.

Plasma proteins are constantly interchanging with tissue proteins. This exchange between these two groups of proteins in the body is described as the "dynamic equilibrium" of plasma and tissue protein.

Nitrogen balance refers to the difference between total nitrogen intake and total nitrogen loss in feces, urine, and perspiration. Positive nitrogen balance is observed when nitrogen intake exceeds the nitrogen output (which is necessary for pregnant women and infants). Normal adult subjects typically are in nitrogen equilibrium. Negative nitrogen balance, where nitrogen output exceeds intake, may occur following surgery, in advanced cancer, and following failure to ingest adequate or sufficiently high-quality protein (e.g. kwashiorkor, marasmus).

SAQ: Mention four factors that affect nitrogen balance.

Ans: Nitrogen balance is affected by negative nitrogen balance caused by infectious diseases (like tuberculosis), surgery, cancer, and hypothyroidism (positive nitrogen balance).

SAQ: What is transamination? What is the significance of transamination?

Ans: The transfer of an amino ($-NH_2$) group from an amino acid to a keto acid by enzyme-catalyzed reaction is known as transamination. At the end of this reaction, two nonessential amino acids are formed.

Example:

$$\text{Alanine} + \text{alpha-ketoglutarate} \xrightleftharpoons{\text{SGPT, Vit } B_6} \text{Glutamic acid} + \text{Pyruvic acid}$$

SGPT: Serum glutamate pyruvate transaminase

Transamination diverts the excess amino acid toward energy generation. For example, the pyruvic acid formed in the above reaction can be converted to glucose by gluconeogenesis, which is the main source of energy.

Q: What is deamination? What is the significance of deamination?

Ans: The removal of an amino group from the amino acids as NH_3 is deamination. It results in the formation of a keto acid and toxic ammonia. Ammonia is converted to urea, and keto acids may participate in transamination. Deamination may be oxidative or nonoxidative.

Q: What is oxidative deamination? What is the significance of oxidative deamination?

Ans: Oxidative deamination takes place mostly in the liver and kidneys. It is the liberation of free ammonia from the amino group of amino acids coupled with oxidation with the formation of alpha-keto acids. The major oxidative deamination of the reaction is catalyzed by the enzyme glutamate dehydrogenase (GLD). It is a mitochondrial enzyme and is most active in the liver.

Most of the amino acids are first transaminated to glutamate, which is then finally deaminated (in the liver).

$$\text{L-glutamate} + \text{NADP}^+ \xrightleftharpoons{\text{GLD}} \text{Alpha-iminoglutarate} + \text{NADPH} + \text{H}^+$$

$$\text{Alpha-iminoglutarate} \xrightleftharpoons{\text{GLD}} \text{Alpha-ketoglutarate} + NH_3 + H_2O$$

Q: What is decarboxylation and the importance of decarboxylation in amino acid metabolism?

Ans: Decarboxylation means the removal of carbon dioxide from an amino acid to form the corresponding amine.

Example: DOPA decarboxylase acts on tyrosine by removing carbon dioxide to form DOPA.

$$\text{Tyrosine} \xrightleftharpoons{\substack{\text{DOPA}\\ \text{decarboxylase}}} \text{dopamine (DOPA)}$$

DOPA is the precursor of the neurotransmitters dopamine, norepinephrine, and epinephrine, collectively known as catecholamines.

Q: What is transmethylation and the importance of transmethylation in amino acid metabolism?

Ans: Transmethylation means the transfer of a "methyl" ($-CH_3$) group from one compound to another compound to form a biochemically useful compound.

Example: Methyl group ($-CH_3$) is transferred from S-adenosylmethionine to guanidinoacetic acid to form creatine.

$$\text{S-adenosylmethionine} + \text{Guanidinoacetic acid} \xrightleftharpoons{\text{Transferase}} \text{S-ademomethylhomocysteine} + \text{Creatine.}$$

Creatine supplies mainly energy to muscles.

SAQ: What is one-carbon metabolism and its importance?

Ans: One-carbon (1C) metabolism means a series of interlinking metabolic pathways that include the methionine and vitamin folic acid involved in biochemical reactions. One carbon units involved are, $-CH_3$, $-CH_2OH$, $-CH$, $-CH{=}O$, etc.

These reactions provide 1C units (methyl groups) for the synthesis of important cellular components such as amino acids, creatine, DNA, polyamines, phospholipids, etc.

Amino acid metabolism is important for the transfer of one-carbon units such as methyl ($-CH_3$), hydroxymethyl (CH_2OH), methenyl ($-CH{=}$), methylene ($=CH_2$), formyl ($-CH{=}O$), forming ($-CH{=}NH$), etc.

Betaine and choline contribute to the formation of coenzyme N5-methyl THF. The coenzyme tetrahydrofolate (THF) actively participates in one-carbon metabolism, which involves S-adenosylmethionine and vitamin B_{12}. Many amino acids act as donors of one-carbon units.

S-adenosylmethionine is a potent aminopropyl and methyl donor that serves as the principal substrate for the methylation of DNA, associated proteins, and RNA.

BAQ: Write a note on glycine biosynthesis.

Ans: Glycine biosynthesis (Fig. 6.15): The main pathway to the synthesis of glycine is a one-step reversible reaction catalyzed by serine hydroxymethyltransferase. This reaction involves the transfer of the hydroxymethyl group from serine to the cofactor tetrahydrofolate (THF), producing glycine and N5, N10-methylene-THF (Fig. 6.15).

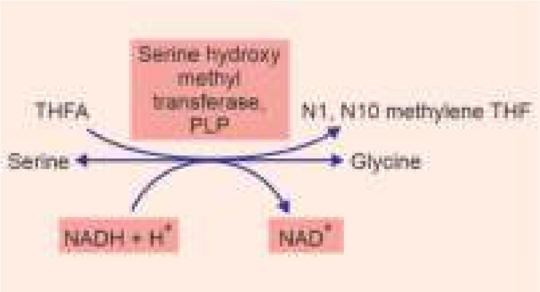

Serine hydroxy methyl transferase, PLP
THFA
N1, N10 methylene THF
Serine
Glycine
NADH + H+
NAD+

Fig. 6.15: Biosynthesis of glycine from serine

BAQ: Enumerate important functions of glycine.

Ans: The following are the important functions of glycine:

1. Glycine is the commonest small amino acid present in protein structure. Collagen contains about 30% content of glycine.
2. Following are the important products formed in the body by using glycine molecules: Purine ring, Glutathione: Glutathione (GSH), bile salts, heme, creatine, and creatinine.

SAQ: What is glycinuria?

Ans: Glycinuria is a rare clinical condition observed due to defective absorption of glycine by renal tubules, although blood glycine level is normal. It may lead to the formation of oxalate stones.

SAQ: What are glutathione and its role in the biochemical reactions of cells?

Ans: Glutathione (GSH) is a tripeptide that contains a specific peptide linkage between the amine group of cysteine (which is attached by a normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, which prevents damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.

BAQ: Write a note on serine biosynthesis.

Ans: The main pathway to the biosynthesis of serine starts with the glycolytic intermediate 3-phosphoglycerate. An NADH-linked phosphoglycerate dehydrogenase converts 3-phosphoglycerate into a keto acid, 3-phosphopyruvate. Aminotransferase activity with glutamate (as a donor) produces 3-phosphoserine, which is converted to serine by phosphoserine phosphatase (Fig. 6.15). Serine can be derived from glycine (and vice versa) by a single-step reaction that involves serine hydroxymethyltransferase and tetrahydrofolate (THF) (Fig. 6.16).

BAQ: Write a note on glutamate biosynthesis and the importance of glutamate as an excitatory neurotransmitter.

Ans: Glutamate is synthesized from its widely distributed alpha-keto acid precursor and ammonium ions by a simple 1-step transamination reaction catalyzed by glutamate dehydrogenase. Glutamate dehydrogenase reaction plays a central role in overall nitrogen homeostasis (Fig. 6.17).

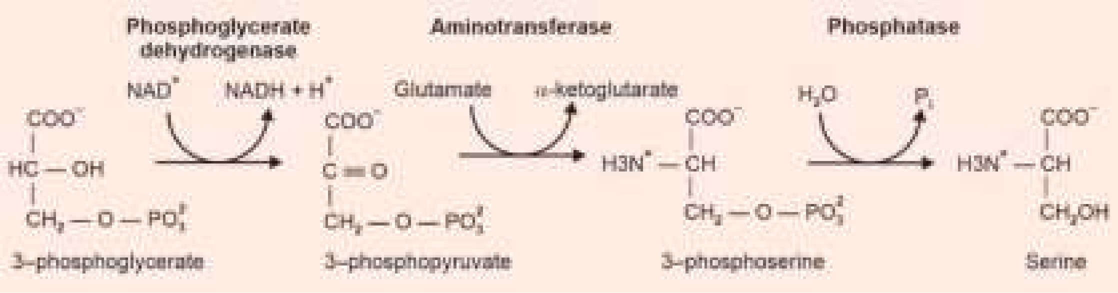

Phosphoglycerate dehydrogenase
Aminotransferase
Phosphatase
NAD+
NADH + H+
Glutamate
α-ketoglutarate
H2O
Pi
COO−
HC — OH
CH2 — O — PO3²−
C = O
H3N+ — CH
CH2OH
3-phosphoglycerate
3-phosphopyruvate
3-phosphoserine
Serine

Fig. 6.16: Serine biosynthesis

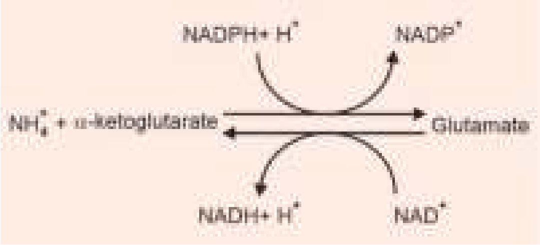


Fig. 6.17: Biosynthesis of glutamate

Glutamate is a precursor for glutamine, proline, arginine, and polyamines.

Glutamate is the most abundant free amino acid in the brain and it takes part in several metabolic pathways. Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate has excitatory effects on nerve cells, and a high concentration of glutamate may excite cells causing the death of neurons. This effect is due to glutamate receptors present on the surface of brain cells. The controlled activity of glutamate transporters in extracellular fluid prevents excessive uptake of glutamate by glutamate receptors. The blood–brain barrier also prevents the brain from glutamate present in the blood. The highest concentrations of glutamate are found in synaptic vesicles in nerve terminals from where it can be released by exocytosis.

BAQ: Write a note on alanine biosynthesis and the importance of alanine in amino acid metabolism.

Ans: Alanine is the second prominent amino acid, next only to glutamine as a circulating amino acid. It serves a unique role in the transfer of nitrogen from peripheral tissue to the liver. Alanine is transferred to the circulation by many tissues, mainly by muscle, in which alanine is formed from pyruvate at a rate proportional to intracellular pyruvate concentration by the following reaction (Fig. 6.18):

Glucose oxidation produces pyruvate, which undergoes transamination to alanine. This reaction is catalyzed by alanine transaminase (ALT).

During periods of fasting, skeletal muscle protein is degraded for energy, and alanine is the major amino acid in the protein. The alanine then enters the bloodstream and is transported to the liver. Within the liver, alanine is converted back to pyruvate which is a source of carbon atoms for gluconeogenesis. The newly formed glucose then enters the blood for delivery back to the muscle.

The amino group transported from the muscle to the liver in the form of alanine is converted to urea in the urea cycle and excreted in urine by the kidneys.

BAQ: What is the alanine-glucose cycle (Cahill cycle)?

Ans: During periods of fasting, skeletal muscle protein is degraded for the energy, required for muscular contraction. Alanine is the major amino acid in the protein. The

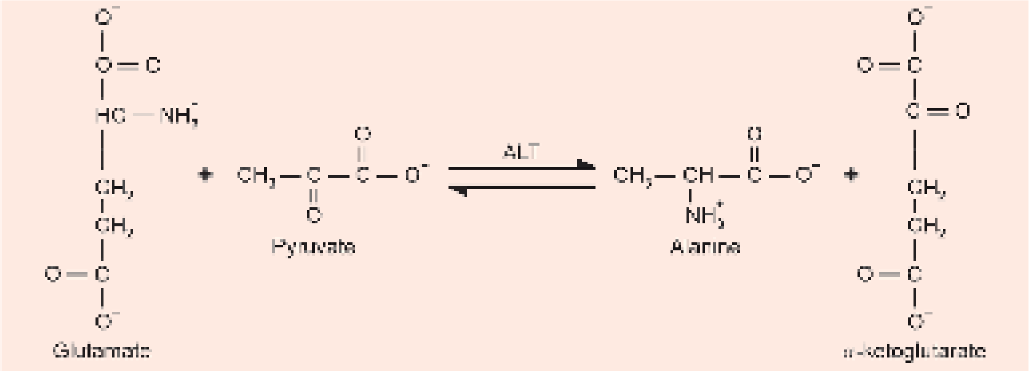


Fig. 6.18: Alanine biosynthesis

alanine then enters the bloodstream and is transported to the liver. Within the liver, alanine is converted back to pyruvate. From pyruvate, glucose is synthesized by gluconeogenesis. The newly formed glucose then enters the blood for delivery back to the muscle for muscular activities. This metabolic transfer of alanine from tissue to liver and transport of glucose obtained from it to tissues is known as the Cahill cycle or alanine–glucose cycle (Fig. 6.19).

The amino group transported from the muscle to the liver in the form of alanine is converted to urea in the urea cycle and excreted in urine by the kidneys.

BAQ: Write a note on the biosynthesis of aspartate and its important metabolic functions.

Aspartate is formed in a transamination reaction catalyzed by aspartate transaminase AST (GOT) between glutamate and oxaloacetate.

D-aspartate plays an important role as a neurotransmitter. It participates in various physiological functions, including important nutritional components, synthesis, and release of glucocorticoids, prolactin, oxytocin, and neuron protection (Fig. 6.20).

BAQ: Write a note on cysteine biosynthesis and its importance in protein synthesis.

Ans: The essential amino acid methionine is required for the synthesis of cysteine. Condensation of methionine and ATP takes place, with the formation of S-adenosylmethionine (SAM). This reaction is catalyzed by methionine adenosyltransferase. (Fig. 6.21).

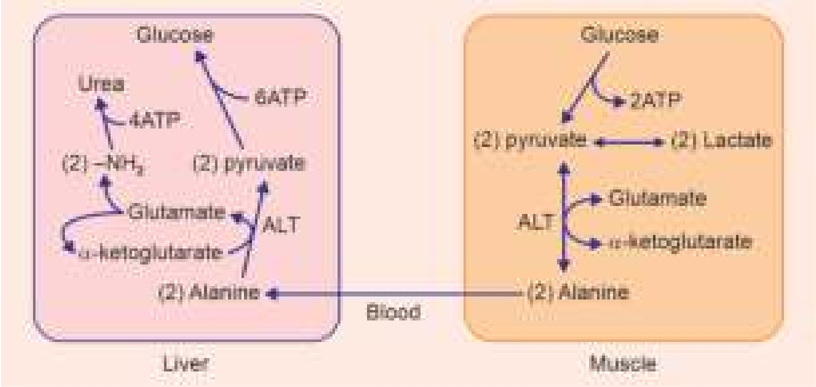

Glucose
Urea
6ATP
4ATP
(2) –NH2
(2) pyruvate
Glutamate
ALT
α-ketoglutarate
(2) Alanine
Liver
Blood
Glucose
2ATP
(2) pyruvate
(2) Lactate
Glutamate
ALT
α-ketoglutarate
(2) Alanine
Muscle

Fig. 6.19: Alanine–glucose cycle

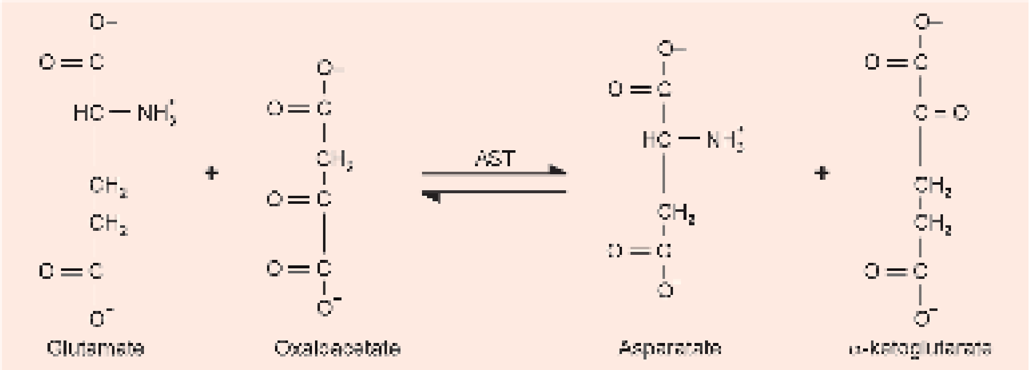

Glutamate
+
Oxaloacetate
AST
Aspartate
+
α-ketoglutarate

Fig. 6.20: Biosynthesis of aspartate

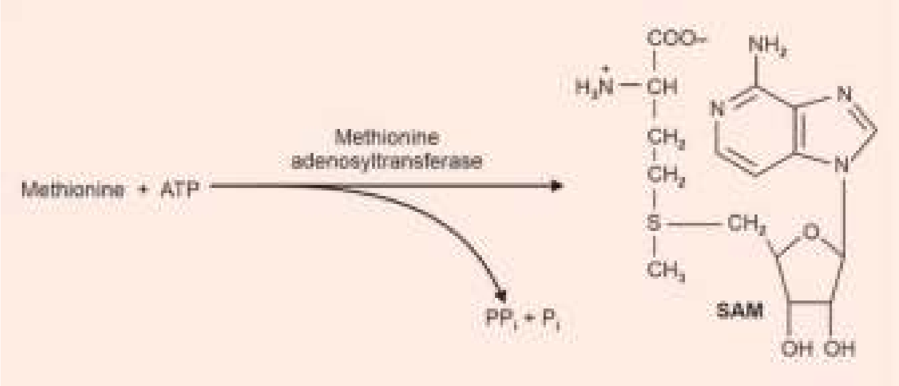


Fig. 6.21: Conversion of methionine to SAM

SAM transfers the methyl group to an acceptor molecule. The result of methyl transfer is the conversion of SAM to S-adenosylhomocysteine, which is then cleaved by adenosylhomocysteinase to yield homocysteine and adenosine.

Homocysteine condenses with serine to produce cystathionine by the action of cystathionine synthase. Cystathionine is subsequently cleaved by cystathionase to produce cysteine and alpha-ketobutyrate. The sum of the latter two reactions is known as trans-sulfuration.

Cysteine readily oxidizes in the air to form the disulfide cystine. Cells contain a small amount of free cystine, since the ubiquitous reducing agent, glutathione, effectively reverses the formation of cystine by a non-enzymatic reduction reaction.

Homocysteine can be converted back to methionine by methionine synthase, a reaction that requires N5-methyl-tetrahydrofolate as a methyl donor.

Cysteine is an important amino acid required for the synthesis of various proteins. It is an important component of beta-keratin, which is the main protein in nails, skin, and hair. Cysteine is necessary for the synthesis of collagen and plays an important role in the folding of the triple helix of collagen. Cysteine can form disulfide bridges that provide strength and rigidity to the keratin protein.

Competency achievement: The student should be able to:

BI5.4: Describe common disorders associated with protein metabolism

BAQ: What is the reason for the increase in homocysteine concentration in blood and its clinical significance?

Ans: Homocysteine condenses with serine to produce cystathionine by the action of cystathionine synthase. Cystathionine is subsequently cleaved by cystathionase to produce cysteine and alpha-ketobutyrate.

Impaired cystathionine synthase due to a genetic defect, leads to increased concentration of homocysteine in blood and homocystinuria takes place (excretion of homocysteine in urine). It is often associated with mental retardation.

Elevated levels of homocysteine in the blood have been shown to correlate with cardiovascular dysfunction. Increased levels of homocysteine in cardiovascular disease are related to its ability to induce a state of inflammation. Homocysteine serves as a negatively charged surface that activates the intrinsic coagulation cascade and leads to inappropriate thrombolytic events causing heart disease.

BAQ: What is cystinuria? What metabolic defect leads to cystinuria? What is the treatment for cystinuria?

Ans: Due to a defect in a specific carrier system in kidney tubules, reabsorption of cysteine, lysine, arginine, and ornithine are affected, and all these amino acids may be present in urine. In "cystinuria", excessive excretion of cysteine takes place. Since cysteine is relatively insoluble in water, increased concentration in urine leads to the formation of cysteine stones in the kidneys.

The treatment for a patient suffering from "cystinuria" includes restricted intake of dietary cysteine.

SAQ: What is cystinosis?

Ans: Cystinosis is a lysosomal storage disease characterized by the abnormal accumulation of the amino acid cysteine. It is a genetic disorder that typically follows an autosomal recessive inheritance pattern. In cystinosis, cysteine crystals are deposited in tissues and organs of the reticuloendothelial system (kidney, liver, spleen, bone marrow, etc.).

BAQ: What is Fanconi syndrome? What is the treatment for Fanconi syndrome?

Ans: Fanconi syndrome is caused by a genetic defect due to which proximal tubules of nephron fail to absorb micronutrients such as glucose, potassium, phosphorus, amino acids like cysteine, uric acid, phosphate, and bicarbonate. Fanconi syndrome also may be acquired, caused by certain drugs and heavy metals. Symptoms of Fanconi syndrome are polyuria, dehydration, muscle weakness, bone weakness, and pain.

Treatment of Fanconi syndrome mainly consists of control of the clinical condition by replacement of fluids and substances that are lost in the urine, mainly electrolytes, bicarbonate, and glucose.

BAQ: Write a note on the biosynthesis of tyrosine and its important metabolic functions.

Ans: Tyrosine biosynthesis takes place as follows: Tyrosine is produced in cells by hydroxylating the essential amino acid phenylalanine. This reaction is catalyzed by the enzyme phenylalanine hydroxylase. Fifty percent of the phenylalanine required goes into the production of tyrosine. If the diet is rich in tyrosine itself, the requirements for phenylalanine are reduced by about 50% (Fig. 6.22).

Tyrosine is a very important component of protein and is used in the synthesis of various neurotransmitters such as DOPA, epinephrine, norepinephrine, and thyroxine

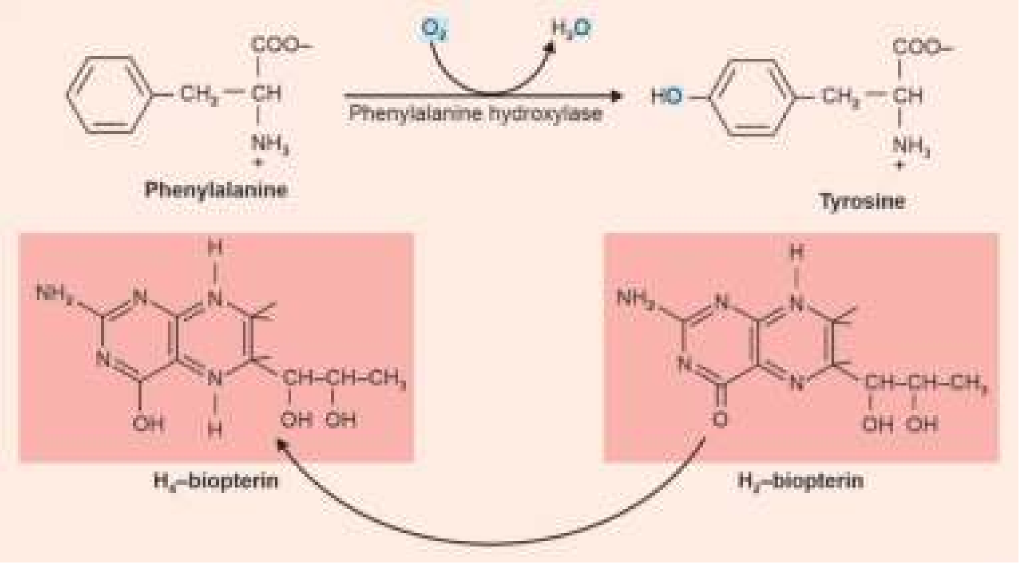


Fig. 6.22: Tyrosine biosynthesis

and it is an important component of melanin, which gives color to eyes, skin, and hair.

INBORN ERRORS OF AMINO ACID METABOLISM

BAQ: Name the inborn error associated with the metabolism of phenylalanine, associated symptoms, how it is managed, and laboratory tests to diagnose it.

Ans: An inborn error leads to a deficiency of phenylalanine hydroxylase. Deficient phenylalanine hydroxylase results in high levels of phenylalanine in the blood—a clinical condition known as hyperphenylalaninemia and excretion of phenylalanine in urine, a clinical condition known as phenylketonuria (PKU). Hyperphenylalaninemia is defined as a plasma phenylalanine concentration greater than 2 mg/dl.

Symptoms of PKU include intellectual disability, hyperactivity, behavioral and social problems, and mental health disorders.

Blood and urine phenylalanine levels are determined to diagnose PKU. Patients suffering from PKU have plasma phenylalanine levels >17–20 mg/dl, whereas non-PKU hyperphenylalaninemia exhibit levels of plasma phenylalanine 17–20 mg/dl.

PKU is managed by providing a patient diet free from phenylalanine.

Serum and urine phenylalanine can be determined by high-pressure liquid chromatography (HPLC).

BAQ: What laboratory techniques are used to determine inborn errors of metabolism related to amino acids?

Ans:

1. Routine urine examination is comprised of physical, chemical, and microscopic examinations. This test will give the general idea of inborn metabolism related to amino acids, by color and odour. In microscope examination, certain amino acids such as cystine can be detected.
2. Paper chromatography and high-performance liquid chromatography (HPLC) is useful to detect specific amino acids in urine.
3. Polymerase chain reaction (PCR) techniques can be used to determine defective genes that cause the inborn metabolism of amino acids.

SAQ: What is the clinical significance of untreated phenylketonuria?

Ans: Untreated PKU leads to severe mental retardation in children caused by the accumulation of phenylalanine, which becomes a major donor of amino groups in aminotransferase activity and depletes neural tissue of alpha-ketoglutarate. The absence of alpha-ketoglutarate in the brain shuts down the TCA cycle and the associated production of aerobic energy, which is essential to normal brain development.

SAQ: Mention three clinical conditions related to degeneration, defect, and lack of melanocytes of the skin.

Ans:

1. Degeneration of melanocytes leads to the formation of leukoderma, leading to the appearance of white patches on the skin.
2. Defects in the melanocytes in the hair roots lead to greying of hair.
3. Albinism is caused due to inborn error with a lack of melanin synthesis.

Q: What is Richner-Hanhart syndrome (tyrosinemia type ll) and its clinical significance?

Ans: Tyrosinemia type ll (Richner-Hanhart syndrome) is caused due to a defect in the enzyme tyrosine transaminase, which leads to a blockade in the catabolism of tyrosine. In this syndrome, accumulation, and excretion of tyrosine and other metabolites such as p-hydrophenylacetate, p-hydrophenylpyruvate, N-acetyltyrosine, tyramine, etc. in urine is observed. Tyrosinemia type ll is characterized by eye lesions, dermatitis, and sometimes mental retardation.

BAQ: Describe maple syrup disease under the following heads:

1. Biochemical basis
2. Clinical features
3. Treatment and
4. Laboratory test for the diagnosis.

Ans:

1. Valine, leucine, and isoleucine (branched-chain amino acids, BCCA) disordered catabolism leads to maple syrup disease. The most common defect is in the branched-chain alpha-keto acid dehydrogenase, which is responsible for converting valine, leucine, and isoleucine to alpha-keto acids. However, due to an inborn deficiency of alpha-keto acid dehydrogenase, all the branch-chain amino acids and related metabolites increase in blood and are excreted in the urine. The main neurological problems are due to the poor formation of myelin in the CNS.
2. The specific symptoms observed in the early stage of the disease are increasing neurological dysfunction and include irritability, lethargy, and poor feeding. These symptoms are followed by focal neurological signs such as increasing spasticity, and abnormal movements, later on, followed by seizures and coma. If untreated, progressive damage to the brain may take place and death occurs usually within weeks or months.
3. Patient suffering from "Maple syrup urine disease" is treated by feeding the patient a diet free from branched amino acids.
4. Maple syrup disease can be diagnosed by determining BCCAs in serum and urine.

   An inborn error can be determined by a PCR test.

Q: Describe alkaptonuria disease under the following heads:

1. Biochemical basis
2. Clinical features
3. Treatment and
4. Laboratory tests for the diagnosis

Ans:

1. Alkatouria is a rare inherited genetic disorder of phenylalanine and tyrosine metabolism. In tyrosine catabolism, one intermediate compound formed is homogentisic acid (HGA). It is further degraded by the enzyme homogentisate 1,2-dioxygenase, an enzyme that converts homogentisic acid (HGA) to maleylacetoacetic acid. Alkaptonuria is caused by a congenital deficiency of homogentisate 1,2-dioxygenase. Homogentisate acid accumulates in the blood and is excreted in the urine in large amounts.
2. Urine in alkaptonuria turns brown or black due to the oxidation of homogentisate to benzoquinone (by polyphenol oxidase), which polymerizes to form dark-colored alkapton.

   Excessive homogentisic acid causes damage to the cartilage (leading to osteoarthritis) and heart valves. Homogentisic acid also precipitates as kidney stones. Alkaptonuria is more common in Slovakia and the Dominican Republic than in other countries. No life-threatening effects accompany this disease. The only untoward consequence of alkaptonuria is ochronosis (bluish-black discoloration of the eyes and ears).
3. Serum and urine specimens can be tested for homogentisic acid by gas chromatography–mass spectroscopy technique.
4. The management of alkaptonuria is palliative, which includes control of pain, physiotherapy, a diet low in tyrosine, a low protein diet, and treatment to reduce the deposition of HGA.

BAQ: Write a note on lysine catabolism.

Ans: α-aminoadipic semialdehyde synthase (AASS) acts on lysine to form saccharopine. This reaction is a transamination type in which the alpha-amino group is transferred to the alpha-keto carbon of alpha-ketoglutarate forming the metabolite, saccharopine. This reaction does not employ pyridoxal phosphate as a cofactor. Saccharopine is immediately hydrolyzed by the enzyme α-aminoadipic semialdehyde synthase (AASS), producing glutamate and α-aminoadipic semialdehyde. The ultimate end-product of lysine catabolism is acetoacetyl-CoA.

BAQ: What are lysinemia and cystinuria?

Ans: The enzyme alpha-aminoadipic semialdehyde synthase (AASS) acts on lysine (in lysine catabolism) and saccharopine forms. Genetic deficiency in the enzyme alpha-aminoadipic semialdehyde synthase (AASS) has been observed in individuals suffering from lysinemia, who excrete large quantities of urinary lysine and saccharopine, leading to cystinuria. The lysinemia and associated cystinuria are benign.

BAQ: Write a note on tryptophan catabolism. How tryptophan is glucogenic as well as ketogenic amino acid?

Ans: The first enzyme of the catabolic pathway of tryptophan is an iron porphyrin oxygenase that opens the indole ring. Kynurenine is the first key branch point intermediate in the catabolic pathway which leads to the following fates:

1. Kynurenine undergoes deamination in a standard transamination reaction yielding kynurenic acid. Kynurenic acid and metabolites have been shown to act as anti-excitotoxic and anticonvulsants. High levels of kynurenic acid have been found in the urine of individuals suffering from schizophrenia. Kynurenic acid has been shown to act as a non-competitive antagonist at the glycine binding site of the NMDA (N-Methyl-D-aspartate) receptor which is an ionotropic (ligand-gated ion channel) receptor for glutamate. The NMDA receptor is an important component of the glutaminergic neurotransmission system believed to be involved in the pathophysiology of schizophrenia.
2. Kynurenine can also undergo a series of catabolic reactions producing 3-hydroxy anthranilic acid and alanine. The production of these alanine residues means tryptophan is a glucogenic amino acid. Oxidation of 3-hydroxy anthranilate leads to the formation of 2-amino-3-carboxymuconic 6-semialdehyde. The main flow of carbon elements from this intermediate leads to acetoacetate; hence, tryptophan is also a ketogenic amino acid.

SAQ: Enumerate three important metabolic compounds formed from tryptophan.

Ans: Tryptophan serves as a precursor for the synthesis of serotonin, melatonin, and niacin.

BAQ: Describe Hartnup disease under the following heads:

1. Biochemical basis
2. Clinical features
3. Treatment
4. Laboratory test for the diagnosis.

Ans:

1. Hartnup disease is an autosomal recessive metabolic hereditary disorder characterized by low plasma levels of tryptophan and urinary excretion of tryptophan and other neutral amino acids. Hartnup disease is caused by the mutation of a specific gene that is responsible for the synthesis of a carrier protein. In Hartnup disease, absorption of tryptophan and other neural amino acids such as valine, glutamate, and alanine, are affected in the absence of the specific carrier proteins.
2. Clinical symptoms of Hartnup disease include mental retardation, dermatitis, ataxia, anxiety, mood swings, sensitivity to light, speech difficulties, abnormalities in muscle tone, short stature, etc.
3. The neutral amino acids such as serine, valine, glutamate, phenylalanine, histidine, alanine, leucine, tyrosine, asparagine, citrulline, isoleucine, threonine, and tryptophan can be detected in the urine of the patient by methods based on chromatography techniques.

4. Treatment is supportive measures with supplements containing appropriate protein with niacin and other B-complex vitamins and avoidance of direct sunlight.

BAQ: Enumerate functions of various hormones in protein metabolism.

Ans:

1. Growth hormone and testosterone are protein anabolic in their action. These hormones increase protein synthesis by producing a decrease in free amino acid levels in the plasma.
2. Effect of insulin is indirect in that it favors glycolysis which in turn provides energy for the formation of peptide bonds.
3. TSH reduces the amino acid content of the blood by favoring anabolic effect on proteins.
4. The metabolic hormones of the adrenal cortex (11-oxy corticosteroids) tend to favor the breakdown of protein and favor catabolism of proteins.

PROTEINURIA

LAQ: Write a note on proteinuria and diagnosis of proteinuria by laboratory tests.

Ans: The glomeruli behave as ultrafilter for the plasma proteins. Normally, high molecular weight proteins such as IgM (MW 900,000) do not appear in glomerular filtrate except in trace amounts. A small amount of albumin (MW 66,000) is passed into the filtrate as a result of its high plasma concentration and relatively low molecular weight. Proteins of 15,000 to 40,000 MW filter more readily but in lesser quantities because of their low plasma concentrations.

The proportions of individual proteins excreted in the urine depend on the extent of their absorption by the renal tubules. Most of the small molecular weight proteins and albumin are reabsorbed by the proximal tubule. Only a small amount of protein is excreted in urine (20 to 150 mg/day); most of it is albumin which is not detected by usual chemical methods in the laboratory. Other proteins excreted in the urine are Tamm-Horsfall protein, an ovomucoid constituent of urinary casts, secreted by distal tubules.

Proteinuria is a clinical condition in which increased amounts of proteins (detectable by usual chemical methods) are present in urine. The following are four ways in which proteinuria can occur.

1. Glomerular proteinuria (due to increased glomerular permeability). In this condition, the urinary protein is mainly albumin. An increase in glomerular permeability occurs in numerous conditions (renal conditions) characterized by diffuse injury to the kidneys.
2. Tubular proteinuria (due to defective tubular reabsorption). In this condition, the urinary proteins are mainly normal low molecular weight plasma proteins. More specific tests are required to detect simple tubular proteinuria since the routine laboratory tests may not detect the presence of proteins in this condition (since albumin may be absent and the dipstick method is specific for albumin).
3. Overload proteinuria (due to increased concentration in the plasma of an abnormal low molecular weight protein). This includes the excretion of Bence Jones proteins, myoglobin, and hemoglobin in urine.
4. Post-renal proteinuria (due to abnormal secretion of proteins in the urine). It may take place due to certain pathological conditions arising from the urinary tract below the kidneys. These include prostate enlargement, inflammation, or malignancy.

Proteinuria observed in certain pre-renal conditions is also known as functional or benign proteinuria. It is associated with exercise, pyrexia, and exposure to cold congestive heart failure, hypertension, or arteriosclerosis.

The following are various clinical features of proteinuria: Edema, observed mainly on the face, and feet of the patient, hypertension, frequent urination, tiredness, nausea, vomiting, lack of appetite, weight loss, etc.

Determination of proteinuria

Protein excretion rate is ordinarily determined from 24-hour urine or a fasting specimen collection since random specimens vary considerably in protein concentration.

In random samples, however, a more convenient way is to determine the protein/creatinine index. Creatinine concentration is relatively constant in any one subject, and the index correlates well with 24-hour total excretion of protein. A ratio greater than 3.5 suggests nephritic-range proteinuria, whereas a ratio of less than 0.2 implies normal total protein excretion.

When 24-hour excretion of protein/creatinine index values is near normal, estimation of one or two individual representative proteins is preferred. When protein excretion exceeds 1 g/day, the proteinuria is likely to be of renal origin (glomerular). When excretion exceeds 2 g/day, heavy loss of plasma albumin may cause generalized edema, as observed in the nephrotic syndrome.

SAQ: What is microalbuminuria?

Ans: Increased urinary albumin excretion in patients with total urinary protein excretion of less than 150 mg/day has been found in the early stages of diabetes mellitus and is termed microalbuminuria.

Q: Describe proteinuria under the following heads:

1. Biochemical basis
2. Clinical features
3. Laboratory test for the diagnosis
4. Treatment

Ans:

1. Proteinuria is a clinical condition in which increased amounts of proteins (detectable by usual chemical methods) are present in urine.
2. Clinical features: Edema observed mainly on face and feet, hypertension, increase in frequency of urination, tiredness, nausea, vomiting, lack of appetite, weight loss, etc.
3. Routine urine examination gives an idea about general functions of kidneys including the functions of nephrons and glomerular basement membrane.
4. Treatment is given according to the clinical presentation, whether it is due to prerenal, renal, or postrenal condition.

Proteins of Muscles

SAQ: Enumerate four names of muscle proteins.

Ans: Actin, myosin, profilin, and troponin

LAQ: Write a note on muscle proteins.

Ans: Two major proteins of muscle are actin and myosin. Monomeric G-actin makes up 25% of muscle protein by weight. In the presence of magnesium ions, G-actin polymerizes noncovalently to form an insoluble double-helical filament called F-actin (Fig. 6.23A).

Profilin is an actin-binding protein involved in the dynamic turnover and restructuring of the actin cytoskeleton. It is found in all eukaryotic organisms in most cells. Profilin is important for spatially and temporally controlled growth of actin microfilaments, which is an essential process in cellular locomotion and cell shape changes. This restructuring of the actin cytoskeleton is essential for important processes such as organ development, wound healing, and the identification of infectious microorganisms by cells of the immune system.

Myosin constitutes a family of various proteins of different types. Myosin II contributes about 55% of muscle protein by weight and forms the thick filament (Fig. 6.23 B). Muscle myosin is myosin II and it is an asymmetric hexamer. It has a fibrous tail consisting of two intertwined helices. Each helix has a globular head portion attached at one end. Myosin hexamer consists of one pair of heavy (H) chains and two pairs

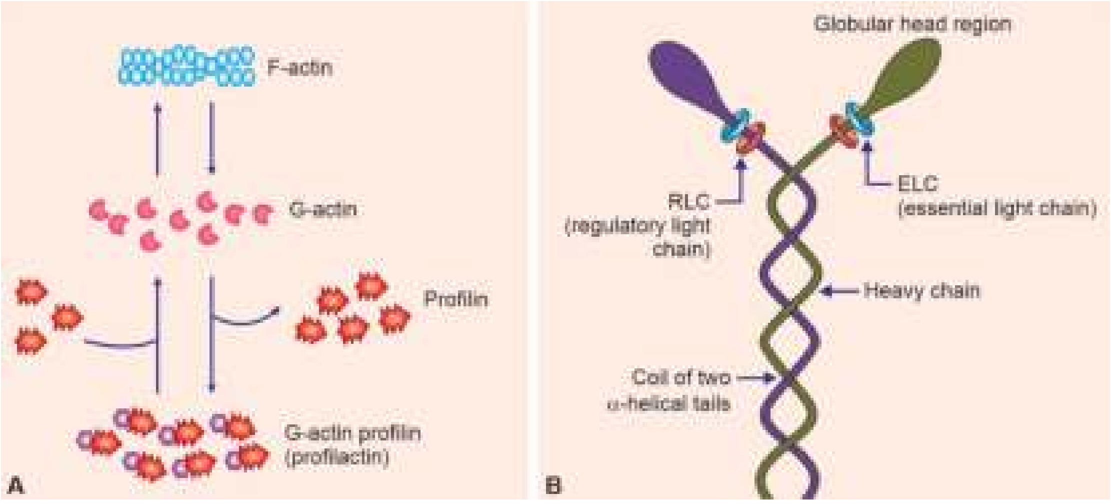

F-actin
G-actin
Profilin
G-actin profilin (profilactin)
A
Globular head region
RLC (regulatory light chain)
ELC (essential light chain)
Heavy chain
Coil of two α-helical tails
B

Fig. 6.23: (A) F-actin, G-actin, profilin and profilactin; (B) Myosin II

of light (L) chains. One light chain is called the essential light chain and the other light chain is called the regulatory chain.

Skeletal muscle myosin binds to actin to form actomyosin and its intrinsic ATPase activity increases significantly. Myosin can only bind to actin when the binding sites on actin are exposed to calcium ions.

Tropomyosin is a two-stranded alpha-helical dimer which is an actin-binding protein. Tropomyosin forms head-to-tail polymers along the length of actin filaments and regulates the access of actin-binding proteins to the filaments (Fig. 6.24).

Troponin (Fig. 6.24) is a complex of three regulatory proteins troponin C, troponin I, and troponin T (TnC, TnI, and TnT). It plays an important role in muscle contraction in skeletal and cardiac muscle, but not in smooth muscle. Troponin is a component of the thin filaments (along with actin and tropomyosin), and is the protein to which

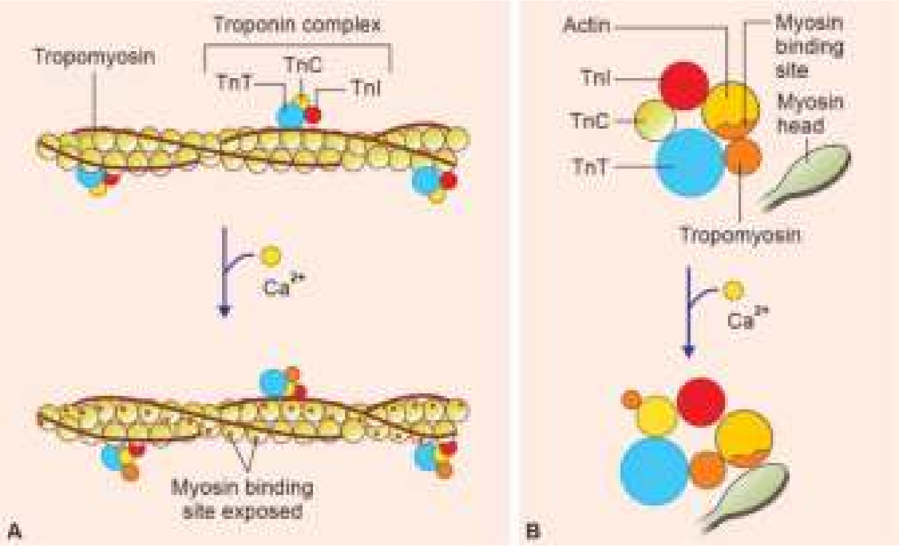

Troponin complex
Tropomyosin
TnC
TnT
TnI
Ca^{2+}
Myosin binding site exposed
A
Actin
Myosin binding site
TnI
TnC
Myosin head
TnT
Tropomyosin
Ca^{2+}
B

Fig. 6.24: Troponin complex and tropomyosin

calcium binds to accomplish regulation of muscular contraction. When calcium is bound to specific sites on TnC, tropomyosin rolls out of the way of the actin filament active sites, so that myosin can attach to the thin filament and produce force and movement. In the absence of calcium, tropomyosin interferes with this action of myosin, and therefore muscles remain relaxed.

BIOCHEMICAL CARDIAC MARKERS

SAQ: Enumerate names of biochemical cardiac markers.

Ans: Cardiac troponins: Serum total troponin (TnT), inhibitory troponin (TnI), myoglobulin, and the isoenzyme of creatine kinase, CK-MB.

BAQ: Write a note on the importance of biochemical cardiac markers.

Ans: Biochemical cardiac markers are serum total troponin (TnT), inhibitory troponin (TnI), myoglobulin, and the isoenzyme of creatine kinase, CK-MB.

Cardiac markers are used for the diagnosis of patients with chest pain and suspected myocardial infarction (MI) and also for the management and prognosis in patients with acute heart failure and pulmonary embolism, etc.

Acute myocardial ischemia represents a spectrum of diseases called acute coronary syndromes, which range from angina, or attacks of thoracic pain, through Q-wave myocardial infarction. The World Health Organization's criteria for diagnosing myocardial infarction are that the patient presents with at least two out of the following three criteria:

1. Clinical symptoms suggestive of myocardial ischemia,
2. Characteristic changes on the electrocardiogram (ECG), and
3. A rise and fall in biochemical markers

The clinical symptoms of MI can be nonspecific for about one-third of the patients, particularly for those with diabetes and for the elderly, who most frequently present atypical symptoms of ischemia. Similarly, the ECG is not a perfect evaluation tool because its clinical sensitivity for MI is only about 50%. Monitoring changes in cardiac markers is considered the benchmark for the diagnosis of MI. Rapid and real-time availability of cardiac markers has become an integral part of most Chest Pain Evaluation Centers (CPEP) protocols, which have cut the rate of missed MI from 4.2% to 0.4%.

LAQ: Write the clinical significance of serum cardiac markers. CK-MB, SGOT, LDH myoglonin, TnT, and TnI.

Ans: CK-MB: When patients experience an MI, the first rise in CK-MB occurs within 4–6 hours after the onset of symptoms. CK-MB remains elevated for about 3 days. It is necessary to monitor serum CK-MB over 8–48 hours, following MI.

SGOT: The increase in SGOT begins 3–8 hours after the onset of the attack and returns to normal in 3–6 days. The highest values are found, on average, some 24 hours after the onset. The duration and extent of the increase is related to the size of the infarct.

LDH: An increase in LDH activity is found beginning within 6–12 hours and reaching a maximum at about 48 hours. The increase is roughly similar to SGOT, but it takes a longer time before normal values are reached again (12–16 days).

Myoglobin: The myoglobin appears earliest in the blood after myocardial injury (30 minutes to one hour). The characteristic early rise of myoglobin is mainly because of its small molecular weight, high concentration in tissue, and renal clearance mechanism.

Cardiac troponins TnT and TnI

Cardiac troponins TnT and TnI present in serum can be used, (1) For the diagnosis of acute MI, (2) For the late diagnosis of MI, and (3) For risk stratification of patients. An increase in serum TnT and TnI is observed within 6 hours after the episode of MI.

For the diagnosis of MI, collection of various blood samples is necessary from 8 to 24 hours. At 12 hours after the onset of symptoms, cTnT had a better clinical sensitivity compared to TnT for the diagnosis of MI. However, the positive serum TnT results are important to identify unstable anginal patients at increased risk for adverse cardiac events.

Troponin T and I remain elevated for a long time, following myocardial infarction (MI), unlike CK-MB. cTnT and cTnI are released for much longer with cTnI detectable in the blood for up to 5 days and cTnT for 7–10 days following MI. This allows an MI to be detected if the patient presents late.

Multiple Choice Questions

Reasons are given for case study questions–answers

Q1. D-amino acids in the body originate from the

A. Racemization of L-amino acids
B. By racemase enzymes
C. By transaminase enzymes
D. A and B

Q2. Which one of the following is a sulfur-containing amino acid?

A. Cysteine B. Leucine
C. Valine D. Alanine

Q3. All the following are sulfur-containing amino acids found in proteins *except*

A. Cysteine B. Tyrosine
C. Cystine D. Methionine

Q4. Which one of the following is an example of essential amino acid?

A. Aspartate B. Glutamate
C. Alanine D. Valine

Q5. The nonessential amino acids

A. Can be synthesized in the body
B. Are not components of blood proteins
C. Are not present in nonvegetarian food
D. Are only present in vegetarian food

Q6. Which of the following is solely a ketogenic amino acid?

A. Threonine B. Leucine
C. Glycine D. Valine

Q7. An amino acid that does not form an α-helix is

A. Proline B. Methionine
C. Serine D. Tryptophan

Q8. In mammalian tissues serine can be a biosynthetic precursor of

A. Glutamic acid B. Glycine
C. Tryptophan D. Methionine

Q9. All the following are the important functions of plasma albumin *except*

A. Transportation of iron
B. Transportation of many required substances
C. In the maintenance of osmotic relations between blood and tissue fluids
D. B and C

Q10. Biuret reagent reacts specifically with

A. Ketogenic amino acids
B. Peptide linkage
C. –S-S- linkage
D. All of the above

Q11. Which peptide acts as a potent smooth muscle hypotensive agent?

A. Bradykinin B. Dopamine
C. Renin D. Glutathione

Q12. Which tripeptide functions as an important reducing agent in the tissues?

A. Bradykinin B. Glutathione
C. C-peptide D. Serotonin

Q13. Which of the following is an example of metalloprotein?

A. Pepsin B. Albumin
C. Globulin D. Ceruloplasmin

Q14. Which of the following is a chromoprotein?

A. Gamma globulin B. Casein
C. Hemoglobin D. Nucleoprotein

Q15. A protein found in the nucleoproteins of the sperm is

A. Gamma globulin B. Ceruloplasmin
C. Protamine D. Albumin

Q16. Which protein is present in hair?

A. Myoglobulin B. Collagen
C. Keratin D. Myosin

Q17. The amino acid from which the synthesis of keratin takes place is

A. Methionine B. Serine
C. Tryptophan D. Hydroxyproline

Q18. In proteins the peptide linkage is an example of

A. Primary structure
B. Secondary structure
C. Tertiary structure
D. Quaternary structure

Q19. At the lowest energy level α-helix of the polypeptide chain is stabilized by the

A. van der Waals forces
B. Hydrogen bonds formed between the H of peptide N and the carbonyl O of the residue
C. Non-polar bonds
D. Disulfide bonds

Q20. Tertiary structure of a protein describes the

A. Specific peptide linkage
B. Location of disulfide bonds
C. Specific way of protein folding and according to the order of amino acids
D. Specific loops

Q21. The technique used for the fractionation of specific proteins is

A. Electrophoresis
B. Ion exchange chromatography
C. Fluorometry
D. ELISA

Q22. Denaturation of proteins leads to

A. Disruption of the primary structure
B. Destruction of hydrogen bonds
C. Fractionation of various types
D. All of the above

Q23. Ceruloplasmin is

A. Gamma globulin B. α_2-globulin
C. Prealbumin D. α_1-globulin

Q24. The end products of protein digestion in GIT are

A. Dipeptides B. Tripeptides
C. Amino acids D. All of the above

Q25. Amino acids are absorbed from the intestine by

A. Facilitated diffusion
B. Passive diffusion
C. Simple diffusion
D. Active transport

Q26. Transamination reactions require which of the following coenzymes?

A. NAD
B. Riboflavin
C. Pyridoxal phosphate
D. NADH

Q27. The main sites for oxidative deamination are

A. Liver B. Intestine
C. Kidneys D. A and C

Q28. A positive nitrogen balance occurs in

A. Diabetes mellitus
B. Growing children
C. Advanced cancer
D. Liver disease

Q29. The main site of urea synthesis is

A. Liver B. Brain
C. Skin D. Intestine

Q30. The enzymes of urea synthesis are found in

A. Mitochondria
B. Cytosol
C. Nucleus
D. Both A and B

Q31. Tryptophan is a precursor of

A. LH B. Epinephrine
C. Melatonin D. Thyroid hormones

Q32. The rate-limiting step in the biosynthesis of catecholamines is

A. Hydroxylation of tryptophan
B. Hydroxylation of tyrosine
C. Oxidation of dopamine
D. Hydroxylation of phenylalanine

Q33. Which amino acid contributes to the detoxification of benzoic acid to form hippuric acid?

A. Alanine B. Serine
C. Methionine D. Glycine

Q34. The main protein of cow's milk is

A. Albumin
B. Casein
C. Gamma globulin
D. Glutein

Q35. Egg does not contain which of the following vitamins?

A. Vitamin C
B. Riboflavin
C. Pantothenic acid
D. Thiamine

Q36. Pulses are rich in which of the following amino acids?

A. Cysteine B. Valine
C. Lysine D. Methionine

Q37. Normal range of serum albumin is

A. 2.0–3.0 g/dl B. 1.0–3.0 g/dl
C. 3.3–4.6 g/dl D. 0.8–1.6 g/dl

Q38. Normal range of total serum proteins is

A. 6.0–8.0 g/dl B. 2.0–4.0 g/dl
C. 3.0–4.0 g/dl D. 4.0–6.0 g/dl

Q39. In serum total proteins the normal range of β globulin is

A. 2.0–4.0 g% B. 1.0–1.8 g%
C. 0.6.–1.2 g% D. 1.2–2.0 g%

Q40. Molecular weight of human albumin is about

A. 180,000 B. 40,000
C. 69,000 D. 125,000

Q41. At isoelectric pH, an amino acid exists as

A. Zwitterion B. Anion
C. Cation D. None of these

Q42. At a pH below the isoelectric point, an amino acid exists as

A. Cation
B. Undissociated molecule
C. Zwitterion
D. Anion

Q43. Which protein is rich in cysteine?

A. Collagen B. Albumin
C. Keratin D. Ceruloplasmin

Q44. Primary structure of proteins can be determined by the use of

A. Electrophoresis
B. Sanger's reagent
C. Chromatography
D. Gel filtration

Q45. The most abundant protein in the body is

A. Elastin B. Collagen
C. Globulin D. Albumin

Q46. Tay-Sachs disease results from an inherited deficiency of

A. Arylsulfatase A
B. A ceramidase
C. Sphingomyelinase
D. Hexosaminidase

Q47. All of the following are required for the synthesis of glutamine *except*

A. Ammonia B. Glutamate
C. NAD D. ATP

Q48. A coenzyme required for the synthesis of glycine from serine is

A. Vitamin B_6 B. ATP
C. NAD D. Tetrahydrofolate

Q49. An organ that is extremely sensitive to ammonia toxicity is

A. Brain B. Heart
C. Kidney D. Liver

Q50. Ammonia is transported from muscles to the liver mainly in the form of

A. Glutamate B. Alanine
C. Asparagine D. A and C

Q51. Histidinemia is caused due to deficiency of

A. Histidine decarboxylase
B. Histidase
C. Histidine carboxylase
D. Histidine oxidase

Q52. Phenylketonuria is caused due to

A. High phenylalanine diet
B. Phenylalanine is not converted into tyrosine
C. Defective tryptophan metabolism
D. A or C

Q53. Histamine is formed from histidine by

A. Decarboxylation
B. Dehydrogenation
C. Carboxylation
D. Deamination

Q54. DOPA is an intermediate in the synthesis of

A. Melanin B. Catecholamines
C. Purines D. Both B and C

Q55. Gastrin stimulates

A. Secretion of HCl
B. Gastric mobility
C. Secretion of bile
D. Both A and B

Q56. Secretin stimulates

A. Secretion of pancreatic juice
B. Inhibition of gastric secretion
C. Secretion of HCl
D. A and C

Q57. Cholecystokinin (pancreozymin) stimulates

A. Contraction of gall bladder
B. Secretion of pancreatic juice
C. Secretion of pepsin
D. A and B

Q58. Which of these are all nonprotein nitrogenous substances?

A. Urea B. Uric acid
C. Creatine D. A, B, C

Q59. After digestion, amino acids are

A. Absorbed into lymph
B. Absorbed into the portal circulation
C. Converted into peptides in the intestine
D. All of the above

Q60. Bence Jones proteins in urine are

A. Precipitated at 40°C
B. Precipitated at 60°C
C. Precipitated at 100°C
D. B and C

Q61. Bence Jones proteins may be excreted in the urine of patients suffering from

A. Diabetes insipidus
B. Prerenal condition
C. Multiple myeloma
D. Hyperthyroidism

Q62. Formation of melanin from tyrosine requires the action of

A. Tyrosinase
B. Diamine oxidase
C. Dopa decarboxylation
D. Glucose 6-phosphatase

Q63. Maple syrup urine disease is due to a deficiency of the enzyme

A. Tyrosinase
B. Phenylalanine hydroxylase
C. Adenosyl transferase
D. Branch chain α-keto acid dehydrogenase

Q64. Alkaptonuria occurs due to a deficiency of the enzyme

A. Branch chain α-keto acid dehydrogenase
B. Homogentisic acid oxidase
C. Dopa decarboxylase
D. Fumarylacetoacetate hydrolase

Q65. Absence of phenylalanine hydroxylase causes

A. Neonatal tyrosinemia
B. Phenylketonuria
C. Primary hyperoxaluria
D. Albinism

Q66. Inherited deficiency of tyrosinase may cause

A. Multiple myeloma
B. Albinism
C. Maple syrup disease
D. Wilson's disease

Q67. A 61-year-old man presented outpatient department of a hospital with pitted edema on both ankles. The attending physician is likely to order the following uristix test:

A. Urine glucose
B. Urine ketone bodies
C. Urine bile pigments
D. Urinary proteins

Q68. An 8-year-old boy reported jaundice, muscle stiffness, behavioral changes, fatigue, and tremors. Examination revealed an enlarged liver and spleen and Kayser ring was noted in the peripheral cornea. Probable diagnosis: The patient was suffering from

A. Viral hepatitis
B. Alcoholic cirrhosis
C. Wilson's disease
D. Acute pancreatitis.

Q69. Which one of the following is not an acute-phase protein?

A. Albumin
B. Fibrinogen
C. Ceruloplasmin
D. C-reactive protein

Q70. The endothelial cells of intact blood vessels prevent blood coagulation by secretion of substances such as

A. Gamma globulin
B. Heparin
C. Thrombomodulin
D. B and C

Q71. Which statement about hemostasis is not correct?

A. Tissue factor combines with factor VIIa in the absence of calcium ions
B. The intrinsic mechanism occurs in response to tissue damage.
C. During clotting, blood cells, and platelets are trapped in a mesh of protein fibers.
D. The extrinsic mechanism is initiated by tissue factors released by damaged tissues.

Q72. Which of the following substance is not correctly matched to its function?

A. Antithrombin-inactivates thrombin
B. Prostacyclin-inhibits platelet activation
C. Heparin-interferes with the formation of prothrombin activator
D. Plasminogen-initiates clot formation

Q73. A patient who lacks fat absorbing mechanism in the intestines is most likely to suffer from severe bleeding problems because

A. Vitamin A will not be absorbed and this will lead to a deficiency of clotting factors
B. Vitamin K will not be absorbed and this will lead to the deficiency of clotting factors
C. Fatty acids are essential for the synthesis of tissue factors
D. Vitamin D will not be absorbed, which is essential for the synthesis of clotting factors.

Q74. Hemophilia A is caused due to the deficiency of the factor

A. I B. V
C. IX D. VIII

Q75. Hemophilia B is caused due to the deficiency of the factor

A. I B. V
C. IX D. VIII

Q76. Following myocardial infarction, CK-MB remains elevated for

A. 1–3 days
B. 4–6 hours
C. 12–16 hours
D. 3–5 days

Q77. For patients with acute coronary syndrome, determination of which of these tests (serum) has been shown to identify patients at risk for adverse events (MI and death)?

A. C-reactive protein B. Troponin T
C. Troponin I D. B and C

Q78. A 54-year-old man presented with malaise and back pain. He lost significant weight in the past three months. In a chemical examination of urine for Bence Jones protein, the report showed a test: positive. The probable diagnosis is, the patient was suffering from

A. Renal disease
B. Multiple myeloma
C. Renal tubular disease
D. A and C

Q79. A 4-year-old boy from the slum area, presented with loss of weight in the past few months, severe edema, abdominal pain, enlarged liver, and diarrhea. There was no indication of jaundice. What is the probable diagnosis?

A. Malnutrition
B. Microbial infection
C. Hepatitis
D. Nephritis

Q80. When serum is compared with plasma, both contain the same components, except the presence of which of the following protein in plasma?

A. α_1 globulin B. β-globulin
C. Fibrinogen D. γ-globulin

Answers

1. D **2.** A **3.** B **4.** D **5.** A **6.** B **7.** A **8.** B **9.** D **10.** B
11. A **12.** B **13.** D **14.** C **15.** C **16.** C **17.** A **18.** A **19.** B **20.** C
21. A **22.** B **23.** D **24.** C **25.** D **26.** C **27.** D **28.** B **29.** A **30.** D
31. C **32.** B **33.** D **34.** B **35.** A **36.** C **37.** C **38.** A **39.** B **40.** C
41. A **42.** A **43.** C **44.** B **45.** B **46.** D **47.** C **48.** D **49.** A **50.** D
51. B **52.** B **53.** A **54.** D **55.** D **56.** D **57.** C **58.** E **59.** D **60.** D
61. C **62.** A **63.** D **64.** B **65.** B **66.** B **69.** A **70.** D **71.** A **72.** D
73. B **74.** D **75.** C **76.** A **77.** D **80.** C

67. D: Pitted edema on both sides of the ankles of the patient indicate low serum proteins and one important reason is a significant loss of protein in urine (proteinuria).

Uristix for protein detects a protein in the urine.

68. C: Wilson's disease, since, symptoms and physical examination observations indicated an enlarged liver and spleen and Kayser-rings were noted in the peripheral cornea of eyes.

78. B: Since Bence Jones proteins are present in urine in multiple myeloma.

79. A: Malnutrition, since the child was not suffering from liver disease and his family status and symptoms indicate "malnutrition" as the primary reason.

Case Studies

Case 1: An eight-year-old boy was admitted to the hospital with generalized edema. His urine was frothy, and a chemical examination revealed massive proteinuria (++++). Laboratory reports were as follows:

	Reference range (Normal range)
Serum total proteins: 3.8 g/dl	6–8 g/dl
Serum albumin: 1.8 g/dl	3.3–4.8 g/dl
Serum globulins: 2.0 g/dl	1.8–3.6 g/dl
Serum albumin/serum globulins: 0.9:1	1.2:1 to 2:2
Urine proteins (fasting): Present +++	Absent

1. What is the probable diagnosis?

Ans: The laboratory test results indicate that serum proteins are lost in the urine, due to damage to the glomerular basement membrane. Significant proteinuria indicates renal disease.

2. What is the mechanism behind generalized edema?

Ans: Due to a significant decrease in serum proteins, a significant amount of water from blood has passed to tissue spaces due to the principle of osmosis. The flow of water is from high water potential to low water potential. Accumulation of water in tissue spaces was indicated by generalized edema.

3. In normal urine proteins are absent. Why protein is present in the urine of this patient?

Ans: Usually high molecular weight proteins are not filtered at the glomeruli and most of the low molecular proteins are reabsorbed by the tubules. If the glomerular basement membrane is damaged by infection or by deposition of antigen–antibody complexes, then low molecular weight albumin leaks first in urine.

4. What is the clinical significance of the changed A/G ratio?

Ans: Serum albumin constitutes about 60% of total proteins. The molecular weight of albumin is lower than globulins. Hence, a large amount of albumin is lost in urine compared to globulins and the ratio of albumin/globulins has changed.

5. What are the additional tests required and what is the probable line of treatment?

Ans: Additional laboratory tests: It is necessary to find out levels of serum urea and serum creatinine to confirm renal condition. For appropriate treatment, it is necessary to find out the cause of renal disease, whether it is due to microbial infection or an autoimmune disorder.

BAQ: Show horizontal integration of Case 1 symptoms and laboratory test values with general medicine, pharmacology and pathology.

Ans: Horizontal integration:

Anatomy: Glomerular damage.

Physiology: Presence of protein in the urine.

Nutrition: Suggestion of appropriate diet for a patient suffering from renal disease.

BAQ: Show vertical integration of Case 1 symptoms and laboratory test values with general medicine and pharmacology.

Ans: Vertical integration:

General medicine: Study of renal disease.

Pharmacology: Study of appropriate drugs to treat and manage renal disease.

Case 2: A middle-aged man was admitted to the hospital following a hematemesis (vomiting of stomach contents, mixed with blood). Case history indicated that he was alcoholic, and had mild jaundice with clinical signs of cirrhosis of the liver. His laboratory test reports were as follows:

	Reference range (Normal range)
Serum total proteins: 5.1 g/dl	6–8 g/dl
Serum albumin: 2.3 g/dl	3.3–4.8 g/dl
Serum globulins: 2.8 g/dl	1.8–3.6 g/dl
Serum albumin/serum globulins: 0.8:1	1.2:1 to 2:2
Urine proteins (fasting): Absent	Absent

1. What is the probable diagnosis?

Ans: The case history indicates that the patient is suffering from a hepatic condition (cirrhosis of the liver) and the laboratory test results indicated that serum total proteins have decreased significantly due to hepatic condition.

2. What is the mechanism behind the decrease in serum proteins?

Ans: Albumin is synthesized in the liver. Due to cirrhosis of the liver (liver disease), the synthesis of albumin is significantly affected, hence total protein levels are decreased.

3. What is the clinical significance of the changed A/G ratio?

Ans: Serum albumin constitutes about 60% of total proteins. Synthesis of albumin is affected due to cirrhosis of the liver (hepatic condition). Hence the ratio of albumin/globulins has changed.

4. What are the additional tests required and what is the probable line of treatment?

Ans: It is necessary to find out levels of serum bilirubin (excretory component by the liver) and liver enzymes such as SGPT, SGOT, and alkaline phosphatase to confirm hepatic condition.

For the appropriate treatment, it is necessary to find the cause of liver cirrhosis whether it is due to excessive alcohol intake, drug abuse, or due to autoimmune disorder.

BAQ: Show horizontal integration of Case 2 symptoms and laboratory test values with anatomy, physiology, and nutrition.

Ans: Horizontal integration:

Anatomy: Liver damage

Physiology: Decrease in serum proteins

Nutrition: Suggestion of appropriate diet for a patient suffering from cirrhosis of the liver.

BAQ: Show vertical integration of Case 2 symptoms and laboratory test values with general medicine and pharmacology.

Ans: Vertical integration:

General medicine: Study of cirrhosis of the liver.

Pharmacology: Study of appropriate drugs to treat and manage cirrhosis of the liver.

Pathology: Study of causes of cirrhosis of liver, pathophysiology and prognosis.

Case 3: A 63-year-old woman presented with back pain and loss of weight. Recently she suffered from frequent chest infections (although she is a non-smoker). She was anemic and suffered from shortness of breath during exercise. Initial laboratory investigation results were as follows:

	Reference range (Normal range)
Serum total proteins: 8.7 g/dl	6–8 g/dl
Serum albumin: 3.8 g/dl	3.3–4.8 g/dl
Serum globulins: 4.9 g/dl	1.8–3.6 g/dl
Albumin/globulins ratio: 1:1.2	1.2:1–2:1.2 g/dl
Urine Bence Jones protein: Present	Absent

1. What is the probable diagnosis?

Ans: Multiple myeloma. The laboratory test results indicate that total serum proteins are increased due to the presence of an abnormal protein called Bence Jones protein.

2. Why A/G ratio has changed?

Ans: Although serum albumin level is normal, globulin fraction is increased due to the presence of additional Bence Jones proteins in serum.

3. What is the probable line of treatment?

Ans: Treatment is given to relieve pain, control complications of the disease, stabilize the clinical condition, and reduce the progress of multiple myeloma. Treatment includes chemotherapy, immunotherapy, radiotherapy, and bone marrow transplant.

BAQ: Show horizontal integration of Case 3 symptoms and laboratory test values with anatomy, physiology, and nutrition.

Ans: Horizontal integration:

Anatomy: A defect in the bone marrow that produces an abnormal number of plasma cells.

Physiology: Increase in serum globulins with the addition of M protein. Hypercalcemia (high blood calcium) due to an increase in bone resorption.

Nutrition: Suggestion of an appropriate diet for the management of multiple myeloma that supports adequate protein and micronutrients, mainly calcium supplements.

BAQ: Show vertical integration of Case 3 symptoms and laboratory test values with general medicine and pharmacology

Ans: Vertical integration:

General medicine: Study of multiple myeloma.

Pharmacology: Study of appropriate drugs to treat and manage multiple myeloma.

Chapter

7

Nonprotein Nitrogenous Molecules

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each
Case Studies: Marks: 4 or 5

INTRODUCTION

When protein is metabolized, about 90 percent of its nitrogen is excreted in the urine in the form of urea, uric acid, creatinine, and other nitrogen end-products. The remaining 10 percent of the nitrogen is eliminated in the feces. A negative nitrogen balance occurs when more protein is used by the body than is taken in. A positive nitrogen balance indicates a net gain of protein in the body. Negative nitrogen balance can be caused by factors such as malnutrition, blood loss, serious diseases, and excessive secretions of glucocorticoids. A positive balance can be caused by the actions of growth hormone, and testosterone and also by exercise.

The term blood nonprotein nitrogen mainly comprises urea, uric acid, creatine, creatinine, and amino acids. Estimation of total blood nonprotein nitrogen (NPN) is commonly undertaken as a measure of protein catabolism or renal function. The normal NPN level is 25–50 mg/dl, and approximately 50% of this is urea nitrogen.

In renal disease, due to the disturbance in the kidney functions and mainly due to decreased glomerular filtration rate, nitrogenous waste products such as urea, creatinine, and uric acid increase in blood. Azotemia means high serum or plasma nonprotein nitrogen.

Total NPN is rarely estimated since a change in plasma urea nitrogen usually adequately reflects both renal retentions of nitrogen and total protein catabolism. The other NPN constituents, such as creatinine and uric acid are estimated individually when required.

Uremia is a clinical syndrome, that occurs when there is marked nitrogen retention due to renal failure. "Uremia" can be defined as an expression of a group of signs and symptoms in patients with severe azotemia secondary to acute or chronic renal failure.

NONPROTEIN NITROGEN

BAQ: Write a short note on nonprotein nitrogen (NPN) and the importance of the determination of NPN.

Ans: The term blood nonprotein nitrogen mainly comprises urea, uric acid, creatine, creatinine, and amino acids. Estimation of total blood nonprotein nitrogen (NPN) is commonly undertaken as a measure of protein catabolism or renal function. The normal NPN level is 25–50 mg/dl, and approximately 50% of this is urea nitrogen. In renal disease, due to the disturbance in the kidney functions and mainly due to decreased glomerular filtration rate, nitrogenous waste products such as urea, creatinine, and uric acid increase in blood. Total NPN is rarely estimated since a change in plasma urea nitrogen usually adequately reflects both renal retention of nitrogen and total protein catabolism. The other NPN constituents, like creatinine and uric acid, are estimated individually to diagnose various clinical conditions.

SAQ: What is azotemia and uremia?

Ans: Azotemia means a significant increase in nonprotein nitrogen in the blood.

Uremia is a clinical syndrome (group of signs or symptoms), that occurs when there is marked nitrogen retention due to renal failure.

UREA METABOLISM

BAQ: Write a brief note on urea metabolism.

Ans: Urea metabolism: The first step towards the metabolic breakdown of amino acids is deamination. The liver is the chief site of deamination, although the kidney and, perhaps, other organs may also accomplish it. Oxidative deamination involves the removal of the alpha-amino group of amino acids to their corresponding keto acids. The enzyme amino acid oxidase carries out the reaction.

$$\text{Amino acids} \xrightarrow[\text{Amino acid oxidase}]{\text{Deamination}} \text{Keto acids} + NH_3$$

The ammonia produced in deamination is toxic. It may be used for (1) the amination of keto acids, (2) excreted as an ammonium salt, and (3) the majority of the ammonia is converted to urea. Thus, urea is the principal end product of amino acid metabolism in the body.

The keto acids may be converted to glucose or fat or maybe catabolized directly to CO_2 and water.

Urea formation takes place mainly in the liver. Two molecules of ammonia and one molecule of CO_2 are converted to urea for each turn of the cycle.

UREA CYCLE

LAQ: Describe reactions and regulation of the Krebs-Henseleit cycle (Urea cycle).

Ans: Urea is the major end product of nitrogen metabolism in humans and mammals. Ammonia, the product of oxidative deamination reactions, is toxic in even small amounts and must be removed from the body. The urea cycle describes the conversion reactions of ammonia into urea. Since these reactions occur in the liver, the urea is then transported to the kidneys for excretion in urine.

The series of reactions that form urea is known as the urea cycle or the Krebs-Henseleit cycle. The urea cycle consists of five reactions: Two mitochondrial and three cytosolic (Fig. 7.1).

The essential features of the urea cycle reactions and their metabolic regulation are as follows:

Deamination of amino acids takes place as follows:

$$\text{Amino acids} \xrightarrow{\text{Amino acid oxidase}} \text{Keto acids} + NH_3$$

Toxic ammonium ions combine with carbon dioxide as shown in reaction 1 of urea cycle.

In the urea cycle the following first two reactions take place in the mitochondria:

1. Ammonium ions combine with CO_2 to form carbamoyl phosphate. This reaction is catalyzed by the enzyme carbamoyl phosphate synthetase (CPS I).
   This reaction is rate-limiting and irreversible.
2. Ornithine transcarbamoylase enzyme catalyzes the condensation of ornithine with carbamoyl phosphate, producing citrulline. Citrulline is then transported to the cytosol via the action of the transporter called citrin, where the remaining reactions of the urea cycle take place.
3. Citrulline and aspartate are condensed to form argininosuccinate. This reaction

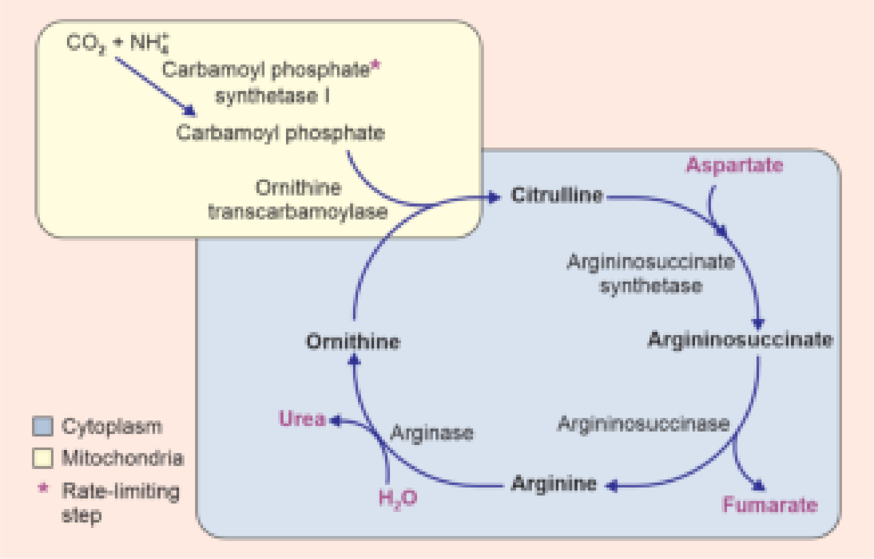

CO_2 + NH_4^+
Carbamoyl phosphate*
synthetase I
Carbamoyl phosphate
Ornithine
transcarbamoylase
Citrulline
Aspartate
Argininosuccinate
synthetase
Argininosuccinate
Ornithine
Urea
Arginase
Argininosuccinase
H_2O
Arginine
Fumarate
Cytoplasm
Mitochondria
* Rate-limiting
step

Fig. 7.1: Reactions of urea cycle

is catalyzed by the cytosolic enzyme argininosuccinate synthetase. This reaction requires ATP.

4. From argininosuccinate, arginine and fumarate are produced by the action of the cytosolic enzyme argininosuccinase.
5. In the final step of the cycle, arginase acts on arginine to form ornithine and urea. Ornithine is transported to the mitochondrial matrix for another round of urea synthesis.

The overall urea formation reaction is:

2 Ammonia + Carbon dioxide + 3 ATP ⟶ Urea + Water + 3 ADP

The reactions of the cycle consume 3 equivalents of ATP and a total of 4 high-energy nucleotide phosphates.

Urea is the only new compound generated by the cycle; all other intermediates and reactants are recycled.

One turn of the cycle: Consumes 2 molecules of ammonia, 1 molecule of carbon dioxide, creates 1 molecule of urea and regenerates one molecule of ornithine for another turn.

Regulation of the urea cycle:

The urea cycle operates only to eliminate excess nitrogen in the form of urea. On high-protein diets, the carbon skeletons of the amino acids are oxidized for energy or stored as fat and glycogen, but the amino nitrogen must be excreted. To facilitate this process, enzymes of the urea cycle are controlled at the gene level. When dietary proteins increase significantly, enzyme concentrations rise. On return to a balanced diet, enzyme levels decrease. Under conditions of starvation, enzyme levels rise as proteins are degraded, and amino acid carbon skeletons are used to provide energy, thus increasing the quantity of nitrogen that must be excreted.

SAQ: Enumerate 5 clinical conditions associated with inherited diseases related to enzymes of the urea cycle.

Ans:

1. Ornithine transcarbamoylase deficiency
2. Carbamoyl phosphate synthetase deficiency
3. Argininosuccinic aciduria
4. Arginemia
5. Citrullinemia

BAQ: Write a note on inherited diseases related to enzymes of the urea cycle.

Ans: High ammonia levels are toxic to humans. A complete block of any step in the urea cycle is fatal since there is no known alternative pathway for the synthesis of urea. Inherited disorders from defective enzymes may cause a partial block in some of the reactions and result in hyperammonemia which can lead to mental retardation. Extensive ammonia accumulation leads to extensive liver damage and death. Inherited diseases of the urea cycle may be caused due to the following reasons, and some of them are associated with hyperammonemia.

1. Ornithine transcarbamoylase deficiency
2. Carbamoyl phosphate synthetase deficiency
3. Argininosuccinic aciduria
4. Argininemia
5. Hyperornithinemia, hyperammonemia, homocitrullinuria syndrome (HHH syndrome)
6. Citrullinemia

LAQ: Explain the routes of ammonia disposal via 1. Glutamate 2. Glutamine and 3. Urea

Ans:

1. Oxidative deamination takes place mostly in the liver and kidneys. It is the liberation of free ammonia from the amino group of amino acids coupled with oxidation with the formation of alpha-keto acids. The major oxidative deamination of the reaction is catalyzed by the enzyme glutamate dehydrogenase (GLD). It is a mitochondrial enzyme and is most active in the liver.

Most of the amino acids are first transaminated to glutamate, which is then finally deaminated (in the liver).

$$\text{L-glutamate} + \text{NADP}^+ \xrightarrow{\text{GLD}} \text{Alpha-iminoglutarate} + \text{NADPH} + \text{H}^+$$

$$\text{Alpha-iminoglutarate} \xrightarrow[\text{H}_2\text{O}]{\text{GLD}} \text{Alpha-ketoglutarate} + \text{NH}_3$$

Ammonia is then converted to urea in the urea cycle and excreted in urine.

The overall urea formation reaction is as follows:

2 Ammonia + Carbon Dioxide + 3 ATP → Urea + Water + 3 ADP

2. Glutaminase is an important kidney tubule enzyme involved in converting glutamine (from the liver and other tissue) to glutamate + NH_4^+ → Urea + α-Ketoglutarate (Fig. 7.2). Ammonium ions are excreted in the urine as ammonium chloride. Glutaminase activity is present in many other tissues, although its

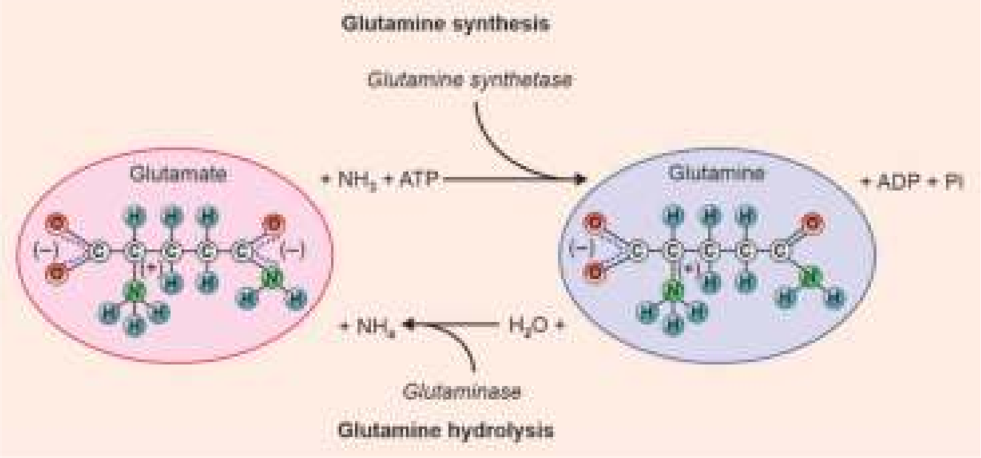


Fig. 7.2: Formation of ammonia in glutamine synthesis and hydrolysis

activity is not as prominent as in the kidney. The glutamate produced from glutamine is converted to alpha-ketoglutarate, making glutamine a glucogenic amino acid.

3. **Urea metabolism:** The first step towards the metabolic breakdown of amino acids is deamination. The liver is the chief site of deamination, although the kidney and, perhaps, other organs may also accomplish it. Oxidative deamination involves the removal of the alpha-amino group of amino acids to their corresponding keto acids. The enzyme amino acid oxidase carries out the reaction.

$$\text{Amino acids} \xrightarrow[\text{}]{\substack{\text{Deamination}\\ \text{Amino acid oxidase}}} \text{Keto acids} + NH_3$$

The ammonia produced in deamination is toxic. It may be used for: (1) The amination of keto acids, (2) it may be excreted as an ammonium salt, and (3) the great majority of the ammonia is converted to urea. Thus urea is the principal end product of protein (amino acid) metabolism in the body.

Urea formation takes place mainly in the liver. Two molecules of ammonia and one molecule of CO_2 are converted to urea for each turn of the cycle.

The concentration of urea in blood plasma represents mainly a balance between urea formation from protein catabolism and urea excretion by the kidneys. Urea is filtered at the glomeruli and after partial reabsorption, it is excreted in the urine. In 24 hours, about 20 to 30 g of urea is excreted, and the normal plasma urea level is 15 to 45 mg/dl.

BAQ: Describe hyperammonemia under the following heads:

1. Biochemical basis
2. Clinical features
3. Diagnostic laboratory test

Ans:

1. Urea is synthesized from ammonia in the liver. In severe liver diseases such as severe viral hepatitis and cirrhosis liver synthesis of urea from ammonia is affected, leading to hyperammonemia, which means significantly high levels of ammonia in the blood,
2. High ammonia levels in the blood may lead to brain damage, coma, followed by death.
3. Quantitative determination of plasma ammonia gives a measure of blood ammonia.

BAQ: Describe azotemia under the following heads:

1. Biochemical basis
2. Clinical features
3. Diagnostic laboratory test.

Ans:

1. **Biochemical basis:** Increase in blood nonprotein nitrogen (NPN). Azotemia means high plasma NPN, and it occurs due to the following various biochemical changes:
   A. *Increased tissue protein catabolism associated with a negative nitrogen balance:* This occurs in fevers, diabetic coma, thyrotoxicosis, bleeding from the gastrointestinal tract, effects of corticosteroids, etc.
   B. Excess breakdown of blood protein as seen in leukemia. The release of leukocyte protein contributes to the high plasma urea. In gastrointestinal disease, plasma proteins and hemoglobin can be released into the gut and digested. This may contribute to high plasma urea.
   C. *Diminished excretion of urea:* This is the most commonest and most important cause of azotemia. It may be due to pre-renal, renal, or post-renal conditions.
2. **Clinical features:** Weakness, confusion, shortness of breath, chest pain, irregular heartbeats, etc.
3. **Diagnostic laboratory test:** Azotemia can be diagnosed by determining serum urea nitrogen. The normal range of serum

urea nitrogen is 7–21 mg/dl. High levels of serum urea nitrogen indicate azotemia.

BAQ: Write information related to the following questions, 1 and 2.

1. What is the normal range of serum urea nitrogen?
2. Clinical significance of serum urea nitrogen.

Ans:

1. Normal range of serum urea nitrogen is 7–21 mg/dl.
2. Clinical significance of serum urea nitrogen test: Elevated levels of urea are observed in pre-renal, renal, and post-renal conditions.

   Pre-renal conditions: Diabetes mellitus, dehydration, cardiac failure, hematemesis, severe burns, high fever, etc.

   Renal conditions: Diseases of kidneys.

   Post-renal conditions: Enlargements of the prostate, stones in the urinary tract, tumor of the bladder.

CREATINE METABOLISM

BAQ: Write a short note on creatine

Ans: Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to all cells in the body, mainly muscle. Creatine is naturally produced in the human body from amino acids, primarily in the kidney and liver. It is transported in the blood for use by muscles. Creatine is not an essential nutrient, as it is manufactured in the human body from L-arginine, glycine, and L-methionine. In humans, approximately 50% of stored creatine originates from food (mainly from meat).

Creatine is present in muscle, brain, and blood in free form as well as in the form of creatine phosphate. Traces of creatine are also present in urine. The resting muscle contains four times more creatine phosphate than ATP. This indicates that creatine phosphate serves as the high-energy phosphate storehouse in muscle. ATP is used as the immediate source of energy for muscle contraction.

Creatinine is largely formed in muscle by the irreversible and non-enzymatic removal of water from creatine phosphate. The formation of creatinine is a preliminary step required for the excretion of most of the creatine.

BAQ: Describe biochemical reactions involved in the synthesis of creatine.

Ans: Three amino acids, glycine, arginine, and methionine, are directly involved in the synthesis of creatine. The rate of creatine synthesis depends upon:

A. The supply of precursor compounds, arginine, glycine, and S-adenosylmethionine and
B. Tissue concentrations of the two enzymes involved in creatine biosynthesis, arginine-glycine transaminase, and guanidinoacetate methyltransferase which catalyze the following reactions 1 and 2, respectively:
   1. $\text{Arginine} + \text{Glycine} \xrightarrow{\text{AGAT}} \text{Ornithine} + \text{Guanidinoacetate}$
   2. $\text{Guanidinoacetate} + \text{S-adenosylmethionine} \xrightarrow{\text{GAMT}} \text{Creatine} + \text{S-adenosylhomocysteine}$

The enzyme AGAT (L-arginine glycine amidinotransferase) is a mitochondrial enzyme responsible for catalyzing the first rate-limiting step of creatine biosynthesis and is primarily expressed in the kidneys and pancreas.

The second enzyme in the pathway (GAMT, guanidinoacetate N-methyltransferase) is primarily expressed in the liver and pancreas.

Creatine, synthesized in the liver and kidney, is transported through the blood and taken up by tissues with high energy demands, such as the brain and skeletal muscle, through an active transport system.

Creatine phosphate formation takes place by the following reaction catalyzed by creatine phosphokinase (CK).

$$\text{Creatine} + \text{ATP} \xrightarrow{\text{CK}} \text{Creatine phosphate}$$

The resting muscle contains four times more creatine phosphate than ATP. This indicates that creatine phosphate serves as the high-energy phosphate storehouse in muscle. ATP is used as the immediate source of energy for muscle contraction.

Q: What is Lohmann's reaction? Describe the importance of Lohmann's reaction.

Ans: Formation of creatine and ATP from creatine-phosphate and ADP by the catalytic action of creatine kinase is known as the Lohmann reaction. It is a reversible reaction.

The supply would be quickly exhausted if creatine phosphate were not available for conversion to ATP (Lohmann reaction). The following reaction takes place in muscle contraction, catalyzed by ATPase and creatinine kinase (CK), respectively:

$$\text{ATP} \xrightarrow{\text{ATPase}} \text{ADP} + H_3PO_4 + 11.5 \text{ kilo cal.}$$

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{ATP} + \text{Creatine}$$

During severe exercise, creatine phosphate stored in the muscle is converted to ATP and creatine. This continues until about one-half of the creatine phosphate is used up, and the muscle becomes exhausted. When muscular exercise ceases, glycogen breakdown continues to produce ATP until the level of creatine phosphate in the muscle is restored to normal.

$$\text{Glycogen} + O_2 \xrightarrow{\text{Glycogenolysis}} CO_2 + H_2O + \text{ATP}$$

$$\text{ATP} + \text{Creatine} \xrightarrow{\text{CK}} \text{ADP} + \text{Creatine phosphate}$$

Thus, the presence of creatine phosphate in muscle gives the muscle a muwch greater supply of readily available energy than the ATP alone can supply.

SAQ: Write the names of two clinical conditions in which the excretion of creatine increases significantly.

Ans: Urine excretion of creatine is extremely low. It is excreted in the urine, particularly in muscular disorders. Increase in serum creatine is often found in myasthenia gravis, muscular atrophies, and the different types of myositis.

SAQ: What is the reason for athletes, bodybuilders, and wrestlers consume creatine supplements?

Ans: Creatine supplements are used by athletes, bodybuilders, wrestlers, sprinters, and others who wish to gain muscle mass, typically consuming 2 to 3 times the amount that could be obtained from a very high protein diet.

BAQ: Write a short note on creatinine metabolism. What are the normal ranges of serum creatinine and 24 hours urinary excretion of creatinine? Write the clinical significance of serum creatinine determination.

Ans: Creatinine is an anhydride of creatine. It is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body, depending on muscle mass. It is an end product of creatine metabolism, and it is a waste product. After formation, it is filtered at the glomeruli and secreted by the tubules, and its excretion in urine per 24 hours is 1.5–3.0 gm.

The normal plasma creatinine level is 0.7-1.7 mg/dl. Serum creatinine increases in renal disease. Elevated levels of serum creatinine are also seen in congestive heart failure, shock, and mechanical obstruction of the urinary tract.

BASIC COMPONENTS OF NUCLEIC ACIDS (Fig. 7.3)

Nucleotides are building units (monomer units) of nucleic acids, such as ribonucleic acid and deoxyribonucleic acid present in a cell. The nitrogen-containing bases present in nucleotides are purines such as adenine and guanine and pyrimidines such as cytosine, uracil, and thymine. The purines and the pyrimidines are utilized for the production of DNA and RNA. These nitrogenous bases are synthesized and linked to a phosphorylated ribose sugar residue, and these units are incorporated into growing strands of new DNA or RNA during replication or transcription. They also perform the following several additional functions:

1. In the form of ATP and GTP they act as energy bonds and coenzymes for specific enzyme systems.
2. In the form of cAMP and cGMP they function as regulatory nucleotides.
3. Nucleotides serve as donors of phosphorylase groups in the form of UDP-sugars, GDP-sugars, ATP and GTP.
4. Cytosine triphosphate and uridine triphosphate are both used in the production of biomolecules.

Purines and Pyrimidines (Fig. 7.3)

Purines consist of a six-membered and a five-membered nitrogen-containing ring fused together. Purines present in nucleic acid are adenine and guanine, which are found in both DNA and RNA. Other purines, such as hypoxanthine and xanthine, are not incorporated into the nucleic acids as they are being synthesized but are important intermediates in the synthesis and degradation of the purine nucleotides.

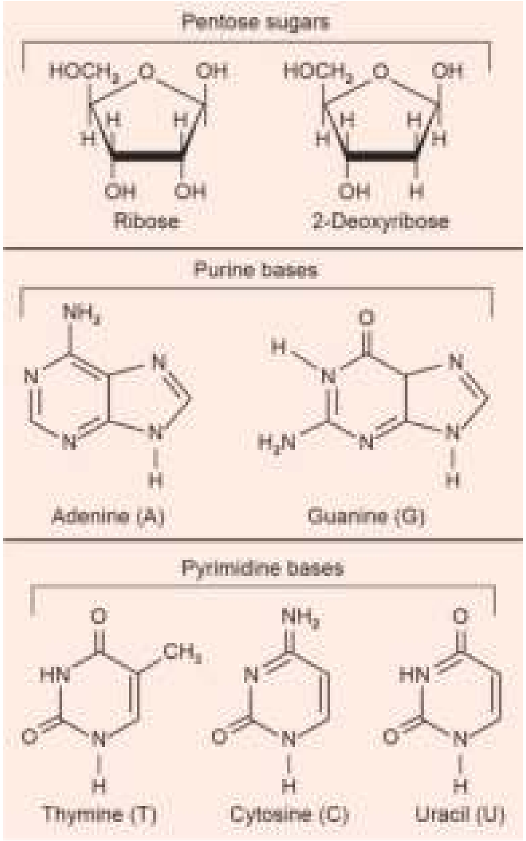


Fig. 7.3: Purines, pyrimidines and pentose sugars

Pyrimidines present in nucleic acid are cytosine, uracil, and thymine. They have only a six-membered nitrogen-containing ring. Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring.

Cytosine is found in both DNA and RNA. Uracil is found only in RNA. Thymine is normally found in DNA. Sometimes tRNA contains some thymine as well as uracil.

Nucleosides and Nucleotides (Fig. 7.4)

If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside. Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or nitrogen 1 of a pyrimidine base. The names of purine nucleosides end in -osine (*Examples:* Adenosine and guanosine) and the names of pyrimidine nucleosides end in idine (*Examples:* Cytidine, uridine, Thymidine). The nucleoside adenosine forms by the combination of adenine and D-ribose. Adding one or more phosphates to the sugar portion of a nucleoside results in a nucleotide.

With different purine and pyrimidines, different nucleotides are formed. For example, guanosine monophosphate (GMP), cytidine monophosphate (CMP), uridine monophosphate (UMP), thymine monophosphate (TMP), etc.

Generally, the phosphate is in ester linkage to carbon 5′ of the sugar. The nucleotide adenosine monophosphate (AMP) is a combination of adenosine and one phosphate group, and the nucleotide adenosine diphosphate (ADP) is a combination of adenosine and two phosphate groups. The

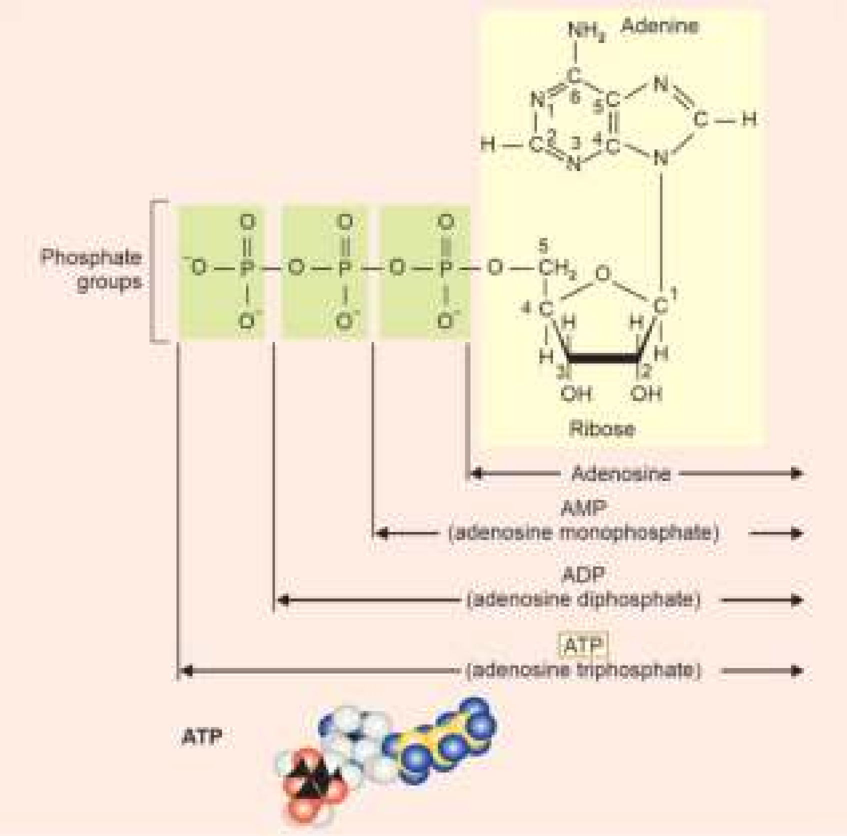

Adenine
Phosphate groups
Ribose
Adenosine
AMP (adenosine monophosphate)
ADP (adenosine diphosphate)
ATP (adenosine triphosphate)
ATP

Fig. 7.4: Nucleotides and nucleosides

nucleotide adenosine triphosphate (ATP) is a combination of adenosine and 3 phosphate groups. Similarly, GMP, GDP, GTP, TMP, TDP, TTP, CMP, CDP, CTP, UMP, UDP, and UTP nucleotides can form using guanine (G), thymine (T), cytosine (C), and uracil (U) bases respectively.

When 2-deoxyribose is a component of a nucleotide, it is written as follows: dAMP, dGMP, dCMP, dTMP, etc.

Nucleotides are joined together by 3′-5′ phosphodiester bonds to form polynucleotides. Polymerization of ribonucleotides will produce an RNA, while polymerization of deoxyribonucleotides leads to the formation of DNA.

In DNA and RNA, the bases form hydrogen bonds with their complementary purines. Thus, the purines adenine (A) and guanine (G) pair up with the pyrimidines thymine (T) and cytosine (C), respectively. In RNA, the complement of A is U instead of T, and the pairs that form are adenine: Uracil and guanine: cytosine.

Nucleic Acids (Figs 7.5 to 7.7)

Nucleic acids are large nitrogenous large molecules present in the cells. These play a very important role in the storage and expression of genomic information. Examples of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

DNA is present in the nucleus of eukaryotic organisms, and it is also found in the mitochondria. DNA is the chemical basis of heredity and may be regarded as the reserve source of genetic information. DNA is exclusively responsible for maintaining the identity of different species of organisms. Every aspect of cellular function is under the control of DNA, which it performs by using specific stretches on it, known as genes. The genes control protein synthesis through the mediation of RNA. **The central dogma** of molecular biology states that DNA contains instructions for protein synthesis, which are copied by RNA, and then these instructions are used to make a specific protein, as shown below:

$$\text{DNA} \xrightarrow{\text{(Transcription)}} \text{mRNA}$$

$$\xrightarrow{\text{(Translation)}} \text{Protein}$$

Deoxynucleic Acid (DNA)

- DNA is a polymer made up of deoxynucleotides. It is composed of monomeric units such as dAMP, dGMP, dCMP, and dTMP.
- The monomeric deoxynucleotides in DNA are held together by 3′, 5′-phosphodiester bridges (Fig. 7.5).
- The DNA is a right-handed double helix (as proposed by **Watson and Crick**). It consists of two antiparallel right-handed double helices in which one strand runs in a 5′5' to 3′3' direction while the other is in a 3′3' to 5′5' direction.
- The two strands are held together by hydrogen bonds formed by complementary base pairs. The A–T pair has 2 hydrogen bonds, while G–C pair has 3 hydrogen bonds (Fig. 7.5).
- In all the species, the DNA has equal numbers of adenine and thymine residues and equal numbers of guanine and cytosine residues.
- DNA double helix is the most predominant form under physiological conditions and is known as **B-form**.
- In a human cell, there are 46 chromosomes (23 pairs) which contain about 100,000 genes.
- A total length of DNA per cell of 1–2 meters is packed into a nucleus, millions of times smaller in diameter.
- An adult human body has about 10^{14} cells. Thus the total length of DNA in the human body is about 2×10^{10} km, i.e. 20 billion km.

Ribonucleic Acid (RNA)

- RNA is a polymer of ribonucleotides held together by 3′, 5′-phosphodiester bridges.

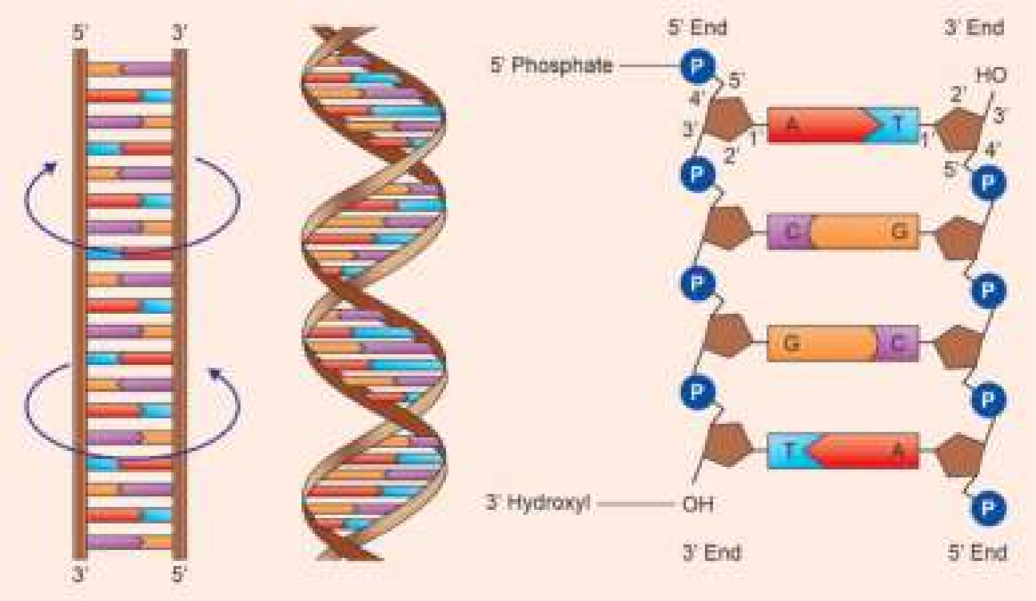


Fig. 7.5: Helical form of DNA and base pairing

- The sugar in RNA is ribose.
- RNA contains the pyrimidine uracil in place of thymine (in DNA).
- RNA is usually a single-stranded polynucleotide. However, this strand may fold at certain places to give a double-stranded structure, if complementary base pairs are nearby.

Following different types of RNAs are present in the nucleus and cytoplasm:

1. mRNA: Messenger RNA is a single-stranded molecule. It is a copy of only one of the two strands of DNA of a gene. It specifies the sequence of amino acids in the protein synthesis (translation).
2. rRNA: Ribosomal RNA is the main site of protein synthesis. They are composed of 60S and 40S subunits. The 60S subunit contains 28S, 5S, and 5.85S subunits, while the 40S subunit contains an 18S subunit. rRNA plays a significant role in the binding of mRNA to ribosomes and protein synthesis (Fig. 7.6).
   60S and 40S subunits combine to form the 80S functional ribosome. The prokaryotic functional ribosome is 70S (a combination of subunits 50S and 30S).
3. tRNA: The presence of several intra-chain hydrogen bonds give tRNA a clover-leaf-like shape (Fig. 7.7). tRNA molecule is folded in the following four loops :

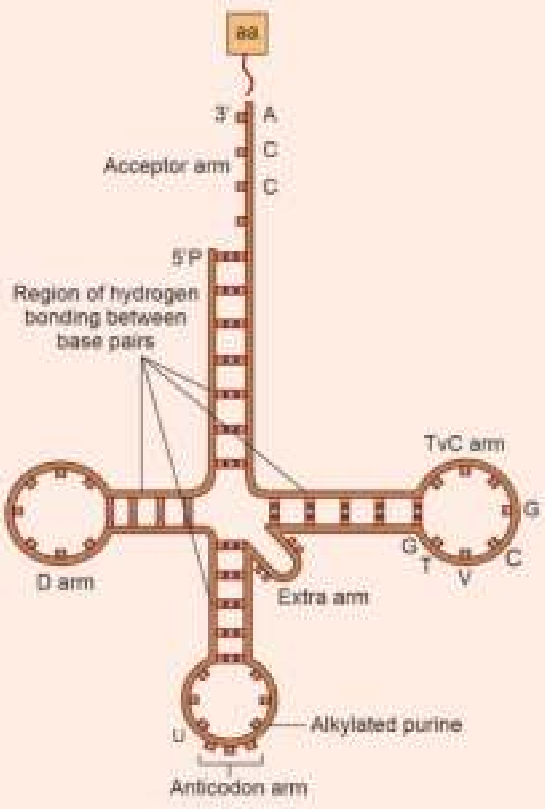

aa
3'
A
C
C
Acceptor arm
5'P
Region of hydrogen bonding between base pairs
TψC arm
G
G
T
ψ
C
D arm
Extra arm
U
Alkylated purine
Anticodon arm

Fig. 7.7: tRNA

1. The D loop and arm: It contains a dihydrouridine (DHU) base. D loop is used for proper recognition of a specific tRNA by its aminoacyl-tRNA synthetase enzyme.

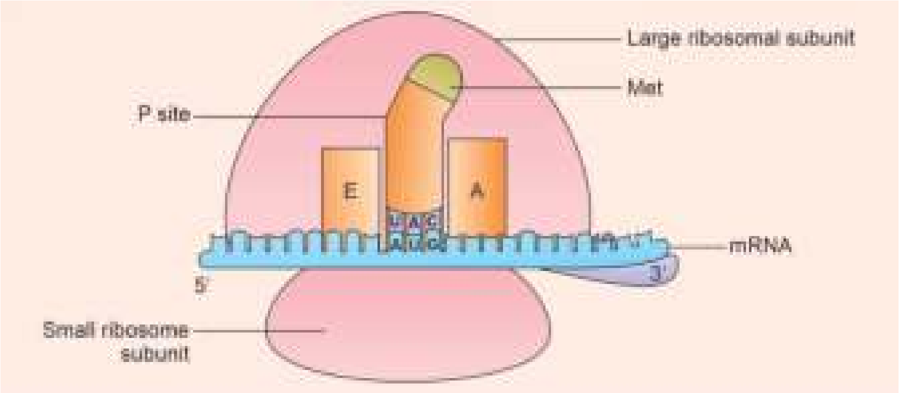

Large ribosomal subunit
Met
P site
E
A
UAC
AUG
mRNA
5'
3'
Small ribosome subunit

Fig. 7.6: Ribosomal RNA

2. The TΨC loop and arm: This loop contains a uracil nucleotide-pseudouridine with a carbon-carbon bond between the sugar and the base. The arm is used in the binding of aminoacyl-tRNA to the ribosome.
3. Anticodon arm: It carries the specific "anticodon" triplet for recognizing the binding site on the ribosome.
4. The variable loop (extra arm): This is present between TΨC arm and the anticodon arm and varies according to a specific tRNA.
5. The 3′-ACC-amino acid acceptor end: It carries a specific amino acid to be transferred into the ribosomes for protein synthesis. About 33 types of tRNA are found in the cytoplasm and about 22 types in mitochondria.

Digestion of Nucleic Acids

Dietary nucleic acids are digested in the small intestine as follows:

1. Digestion of ingested nucleic acids occurs through the concerted actions of pancreatic endonucleases, phosphodiesterases, and nucleoside phosphorylases in the ileum.
2. Endonucleases degrade DNA and RNA at internal sites leading to the production of oligonucleotides.
3. Oligonucleotides are further digested by phosphodiesterases that act from the ends inward, yielding free nucleosides. The nucleotides are hydrolyzed by nucleotidases to give the nucleosides and Pi.
4. The nucleosides are hydrolyzed by nucleosidases that yield ribose-1-phosphate and free bases, which are absorbed in the small intestine and appear in blood circulation.
5. If the nucleosides and (or) bases are not re-utilized, the purine bases are further degraded to uric acid, and the pyrimidines are degraded to β-amino isobutyrate, NH_3, and CO_2.
6. Tissue nucleoproteins are degraded by lysosomal enzymes. After the dissociation of the protein and nucleic acid, the protein is metabolized like any other protein.
7. The purine and pyrimidine bases released are either degraded or salvaged for reincorporation into nucleotides. There is significant turnover of all kinds of RNA as well as the nucleotide pool. DNA does not turnover, but portions of the molecule are excised as part of a repair process.

Competency achievement: The student should be able to:

BI6.2: Describe and discuss the metabolic processes in which nucleotides are involved

BAQ: Describe the importance of purines and pyrimidines. How body cells get purines and pyrimidines?

Ans: Purines and pyrimidines are important for many reasons. Nucleotides are part of nucleic acids. However, other functions of nucleoproteins are as follows: ATP is the most commonly used source of energy. Many other nucleotides are the sources of energy that drive several biochemical reactions. GTP is used in protein synthesis as well as in a few other reactions. UTP is the source of energy for activating glucose and galactose. CTP is an energy source in lipid metabolism. AMP is part of the structure of some of the coenzymes like NAD and coenzyme A.

The bases of nucleotides are nonessential dietary components. These can be synthesized in the body.

The metabolic requirements for the nucleotides and their related bases can be met by both dietary intake or synthesis *de novo* from low molecular weight precursors such as the amino acids, glycine, glutamine, aspartate, and tetrahydrofolate derivatives. The ability to salvage nucleotides from sources within the body means the purine and pyrimidine bases are not required in the diet. The salvage pathways are a major source of nucleotides for the synthesis of DNA, RNA, and enzyme co-factors. The major site of purine synthesis is in the liver. Pyrimidine synthesis takes place in the cytoplasm of the cells.

BIOSYNTHESIS OF PURINES

LAQ: Describe reactions of the biosynthesis of purines.

Ans: The biosynthesis of purines takes place as follows (Figs 7.8 and 7.9):

The major site of purine synthesis is in the liver. Both the salvage and *de novo* synthesis pathways of purine and pyrimidine biosynthesis lead to the production of nucleoside-5′5'-phosphates through the utilization of an activated sugar intermediate and a specific class of enzymes called phosphoribosyltransferases.

- Ribose 5-phosphate is both a product and an intermediate of the pentose phosphate pathway. The purines are synthesized as the ribonucleotides. A necessary prerequisite is the synthesis of the activated form of ribose 5-phosphate. Ribose 5-phosphate reacts with ATP to form 5-phosphoribosyl-1-pyrophosphate (PRPP). This reaction occurs in many tissues because PRPP plays several roles, such as purine and pyrimidine nucleotide synthesis, maintaining salvage pathways, NAD and NADP formation, etc.
- PRPP is generated by the action of PRPP synthetase and requires energy in the form of ATP, as shown in the following reaction given in Fig. 7.8. This reaction requires ATP molecules and the release of AMP molecules to take place.
- Synthesis of the purine nucleotides begins with PRPP and leads to the first fully formed nucleotide, inosine 5′5'-monophosphate (IMP)), through a series of reactions utilizing ATP, tetrahydrofolate (THF) derivatives, glutamine, glycine, and aspartate. The rate-limiting reaction is catalyzed by glutamine PRPP amidotransferase (Fig. 7.9).
- The synthesis of IMP requires five moles of ATP, two moles of glutamine, one mole of glycine, one mole of CO_2, one mole of aspartate, and two moles of formate. The formyl moieties are carried on tetrahydrofolate (THF) in the form of N5, N10-methenyl-THF, and N10-formyl-THF.
- IMP represents a branch point for purine biosynthesis because it can be converted into either AMP or GMP through two distinct reaction pathways.
- The pathway leading to AMP requires energy in the form of GTP;
- The pathway leading to GMP requires energy in the form of ATP.
- The utilization of GTP in the pathway to AMP synthesis allows the cell to control the proportions of AMP and GMP to near equivalence.

There are definite tissue differences in the ability to carry out *de novo* synthesis of purines and pyrimidines. *De novo* synthesis of purines is most active in the liver. Non-hepatic tissues generally have limited or no *de novo* synthesis.

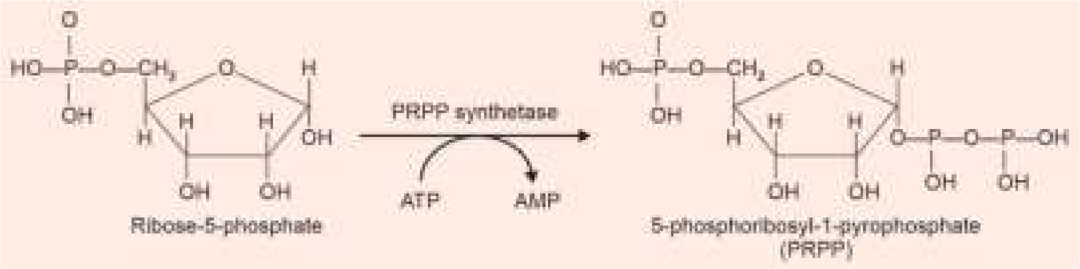


Fig. 7.8: Synthesis of PRPP

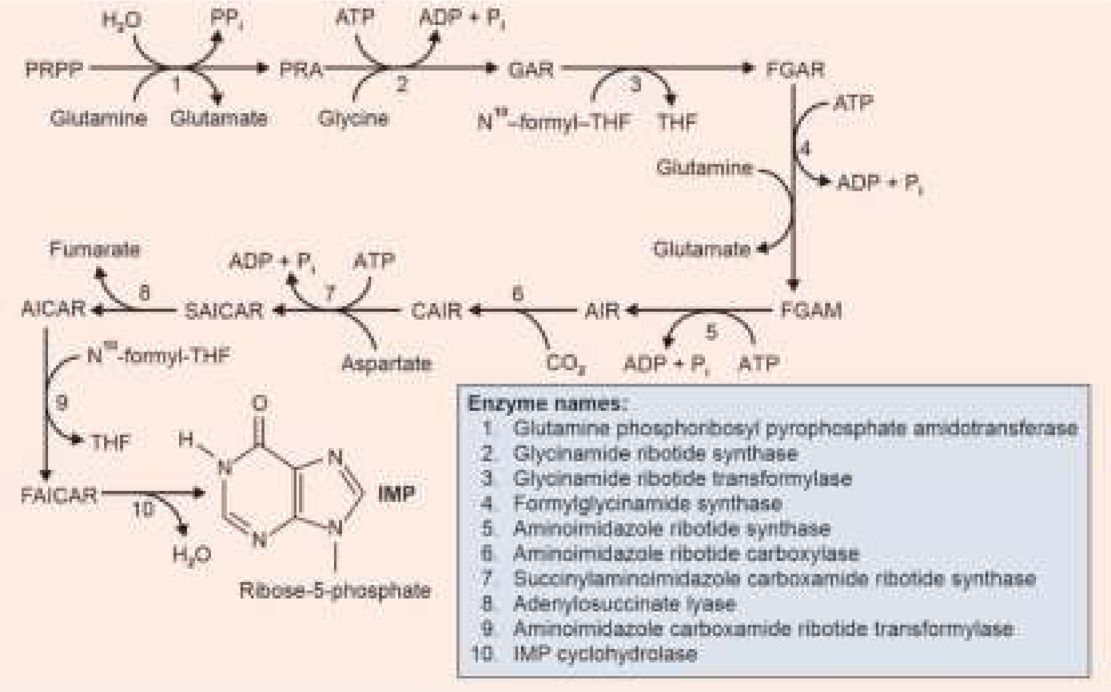

H2O
PPi
ATP
ADP + Pi
PRPP
1
PRA
2
GAR
3
FGAR
Glutamine
Glutamate
Glycine
N10-formyl-THF
THF
ATP
4
Glutamine
ADP + Pi
Glutamate
Fumarate
ADP + Pi
ATP
8
7
6
AICAR
SAICAR
CAIR
AIR
FGAM
5
N10-formyl-THF
Aspartate
CO2
ADP + Pi
ATP
9
THF
O
H
N
N
N
N
IMP
FAICAR
10
H2O
Ribose-5-phosphate
Enzyme names:
1. Glutamine phosphoribosyl pyrophosphate amidotransferase
2. Glycinamide ribotide synthase
3. Glycinamide ribotide transformylase
4. Formylglycinamide synthase
5. Aminoimidazole ribotide synthase
6. Aminoimidazole ribotide carboxylase
7. Succinylaminoimidazole carboxamide ribotide synthase
8. Adenylosuccinate lyase
9. Aminoimidazole carboxamide ribotide transformylase
10. IMP cyclohydrolase

Fig. 7.9: Synthesis of IMP

SAQ: What is the pharmacotherapeutic importance of modulation of purine metabolism by the drug azathioprine?

Ans: Purine synthesis inhibitor such as azathioprine inhibits the proliferation of cells, especially leukocytes. Azathioprine is an immunosuppressant used in organ transplantation, autoimmune diseases such as rheumatoid arthritis, or inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.

SAQ: What is the pharmacotherapeutic importance of modulation of purine metabolism by the drug mycophenolate?

Ans: Mycophenolate mofetil is an immunosuppressant drug used to prevent rejection in organ transplantation. It inhibits purine synthesis by blocking inositol monophosphate dehydrogenase. The drug methotrexate indirectly inhibits purine synthesis by blocking the metabolism of folic acid. It is an inhibitor of the dihydrofolate reductase.

BIOSYNTHESIS OF PYRIMIDINE

LAQ: Write a note on the biosynthesis of pyrimidine.

Ans: Pyrimidine synthesis occurs in a variety of tissues. Unlike in purine biosynthesis, the pyrimidine ring is synthesized before it is conjugated to PRPP.

The biosynthesis of pyrimidine takes place by the following reactions (Fig. 7.10):

- The first reaction is the conjugation of carbamoyl phosphate and aspartate to make N-carbamoyl aspartate. The enzyme that carries out the reaction is aspartate transcarbamoylase, an enzyme that is closely regulated.
- The carbamoyl phosphate synthetase used in pyrimidine biosynthesis is located in the cytoplasm, in contrast to the carbamoyl phosphate used in urea synthesis, which is synthesized in the mitochondrion.
- The second reaction is ring closure to form dihydro-orotic acid by the enzyme dihydro-orotase. This circular product

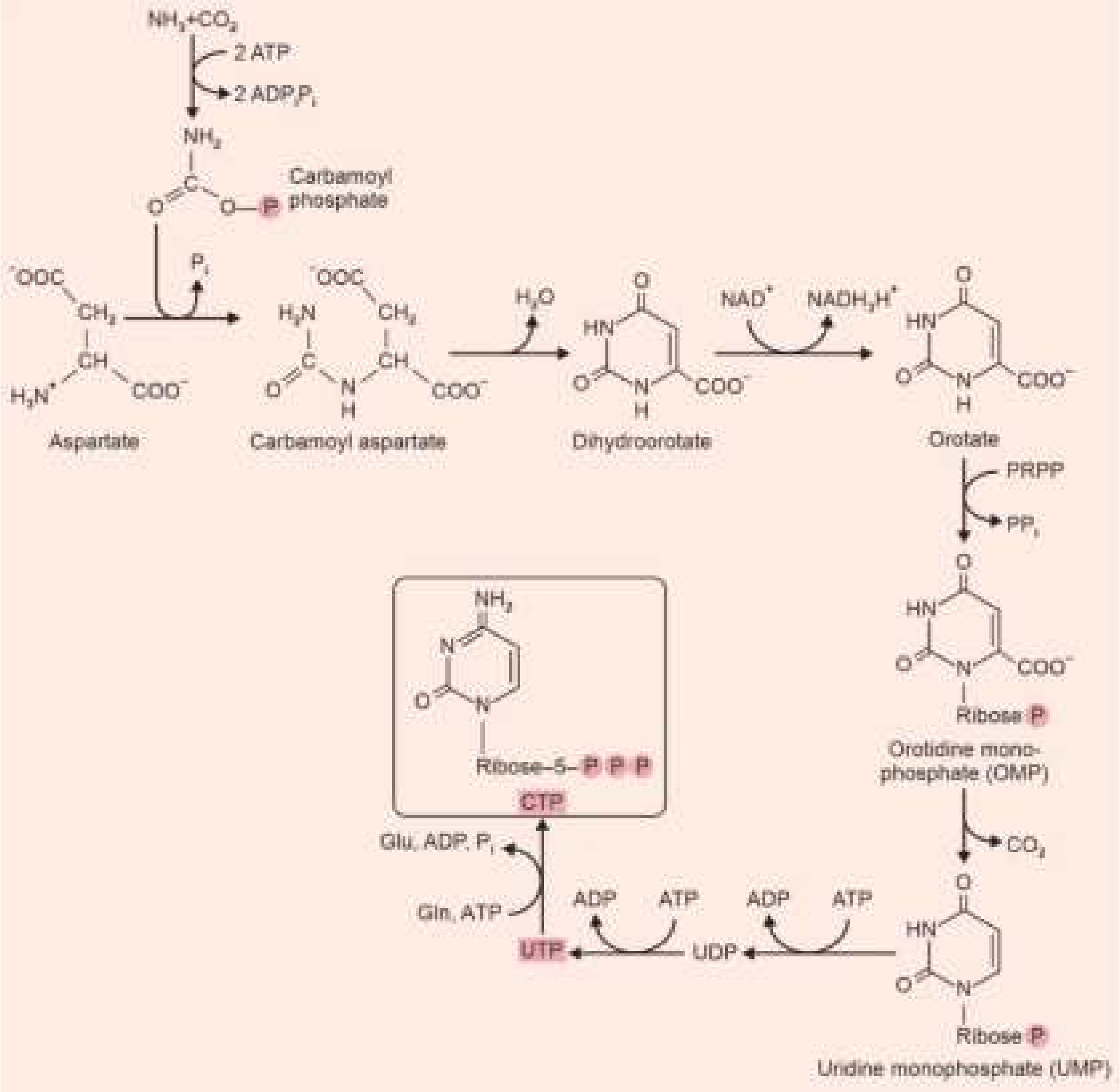

NH3+CO2
2 ATP
2 ADP,Pi
NH2
C
O
O—P
Carbamoyl phosphate
Pi
-OOC
CH2
CH
H3N+
COO-
Aspartate
-OOC
H2N
CH2
C
CH
O
N
H
COO-
Carbamoyl aspartate
H2O
O
HN
O
N
H
COO-
Dihydroorotate
NAD+
NADH,H+
O
HN
O
N
H
COO-
Orotate
PRPP
PPi
O
HN
O
N
COO-
Ribose P
Orotidine monophosphate (OMP)
CO2
O
HN
O
N
Ribose P
Uridine monophosphate (UMP)
ADP
ATP
ADP
ATP
UDP
UTP
Gln, ATP
Glu, ADP, Pi
NH2
N
O
N
Ribose–5–P P P
CTP

Fig. 7.10: Basic steps of the synthesis of pyrimidines

contains a 6-membered ring with nitrogen and carbons located in the same positions as in the mature pyrimidine ring.

- The third reaction is the oxidation of the ring to form a carbon-carbon bond. The reducing equivalents are transferred to a flavin cofactor of the enzyme dihydroorotate dehydrogenase. The product is orotic acid.
- In the fourth reaction, the orotate ring is transferred to phosphoribosyl pyrophosphate (PRPP) to form a 5′ ribose-phosphate, orotidylic acid.
- Finally, orotidylate is decarboxylated to yield UMP, which contains one of the bases of RNA.
- Cellular kinases convert UMP to UTP.
- Transfer of amido nitrogen from glutamine by CTP synthetase converts UTP to CTP. This reaction uses an ATP high-energy phosphate.

Interconversion of Nucleotides

The monophosphates are the forms synthesized *de novo*, although the triphosphates are the most commonly used forms. There are several enzymes classified as nucleoside monophosphate kinases which catalyze the following general reaction:

$$\text{Base-monophosphate} + \text{ATP} \longrightarrow \text{Base-diphosphate} + \text{ADP}$$

Example: Adenylate kinase catalyzes the following reaction:

$$\text{AMP} + \text{ATP} \longrightarrow 2\text{ADP}$$

Similarly, the diphosphates are converted to the triphosphates by nucleoside diphosphate kinase:

$$\text{BDP} + \text{ATP} \longrightarrow \text{BTP} + \text{ADP}$$

SAQ: What is the significance of the salvage of purine and pyrimidine bases?

Ans: Salvaging means saving purine and pyrimidine bases is an exceedingly important process for most tissues. Conversion of purines, related nucleosides, and nucleotides involves "salvage reactions" that require far less energy than *de novo* synthesis. The liver is the major site of purine nucleotide biosynthesis and provides purines and purine nucleosides for "salvage reactions" and for utilization by those tissues, which are incapable of their biosynthesis. The human brain has a low level of phosphoribosyl pyrophosphate (PRPP) glutamyl amidotransferase; hence it depends in part on exogenous purines. Polymorphonuclear leukocytes and red blood cells cannot synthesize 5-phosphoribosyl amine, hence these are dependent on exogenous purines to form nucleotides.

BAQ: Write a description of biochemical reactions involved in the salvage of purine and pyrimidine bases.

Ans: Two distinct pathways possible for salvaging purines and pyrimidines are as follows:

Salvaging Purines

The more important step of the pathways for salvaging purines uses enzymes called phosphoribosyltransferases (PRTs). PRTs catalyze the addition of ribose 5-phosphate to the base from phosphoribosyl pyrophosphate (PRPP) to yield a nucleotide as follows:

$$\text{Base} + \text{PRPP} \longrightarrow \text{Base-ribose-phosphate (BMP)} + \text{PPi}$$

Salvaging Pyrimidines

This second type of salvage pathway involves two steps and is the major pathway for the pyrimidines, uracil, and thymine:

1. Base + Ribose 1-phosphate $\longrightarrow$ Nucleoside + Pi.

   This reaction is catalyzed by nucleoside phosphorylase.
2. Nucleoside + ATP $\longrightarrow$ Nucleotide + ADP. This reaction is catalyzed by nucleoside kinase.

It is important to know the following:

1. The enzyme HG-PRT is very important and it is inhibited by both IMP and GMP. This enzyme salvages guanine directly and adenine indirectly.
2. AMP is generated primarily from IMP.

Competency achievement: The student should be able to:

BI6.4: Discuss the analytes associated with gout and Lesch-Nyhan syndrome

SAQ: Write a note on Lesch-Nyhan syndrome.

Ans: Lesch-Nyhan syndrome is a rare inborn error of purine metabolism characterized by the absence or deficiency of the activity of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). Due to the deficiency of HPRT, the purines hypoxanthine and guanine are not used in the synthesis of nucleotides. The end product of hypoxanthine and guanine metabolism is uric acid. Hence very high levels of serum uric acid levels are observed in Lesch-Nyhan syndrome patients. In this clinical condition, sodium urate crystals may abnormally accumulate in the joints and kidneys.

The symptoms of Lesch-Nyhan syndrome include acute gouty arthritis, impaired kidney function, self-mutilating behaviors, and neurological impairment.

SAQ: Why deficiency of folic acid takes place in the treatment of cancer using chemotherapeutic agents like methotrexate and aminopterin?

Ans: Cancer chemotherapeutic agents like methotrexate and aminopterin are structural analogs of folic acid and inhibit dihydrofolate reductase. This interferes with the maintenance of the folate pool and, thus, of *de novo* synthesis of purine nucleotides and dTMP synthesis. These drugs are highly toxic and administered under careful control.

PURINE CATABOLISM, URIC ACID METABOLISM

Q. Describe biochemical reactions of uric acid metabolism.

Ans: The end product of purine catabolism in humans is uric acid. Other mammals have the enzyme urate oxidase and excrete the more soluble allantoin as the end product. Humans do not have this enzyme; hence, uric acid is an end product of purine metabolism. The following are various reactions involved in uric acid metabolism (Fig. 7.11):

1. Adenosine is deaminated to inosine by an adenosine deaminase.
2. Purine nucleoside phosphorylase acts on inosine and hypoxanthine forms.
3. Xanthine oxidase first acts on hypoxanthine to form xanthine.
4. Xanthine oxidase then acts on xanthine to form uric acid.
5. Guanine nucleotides are hydrolyzed to the nucleoside guanosine, which undergoes phosphorolysis to guanine and ribose 1-P.
6. Guanine deaminase acts on guanine to form xanthine. Xanthine oxidase acts on xanthine to form uric acid.

Uric acid is filtered in the glomeruli and partially reabsorbed by the tubules, and then it is excreted in the urine. The quantity

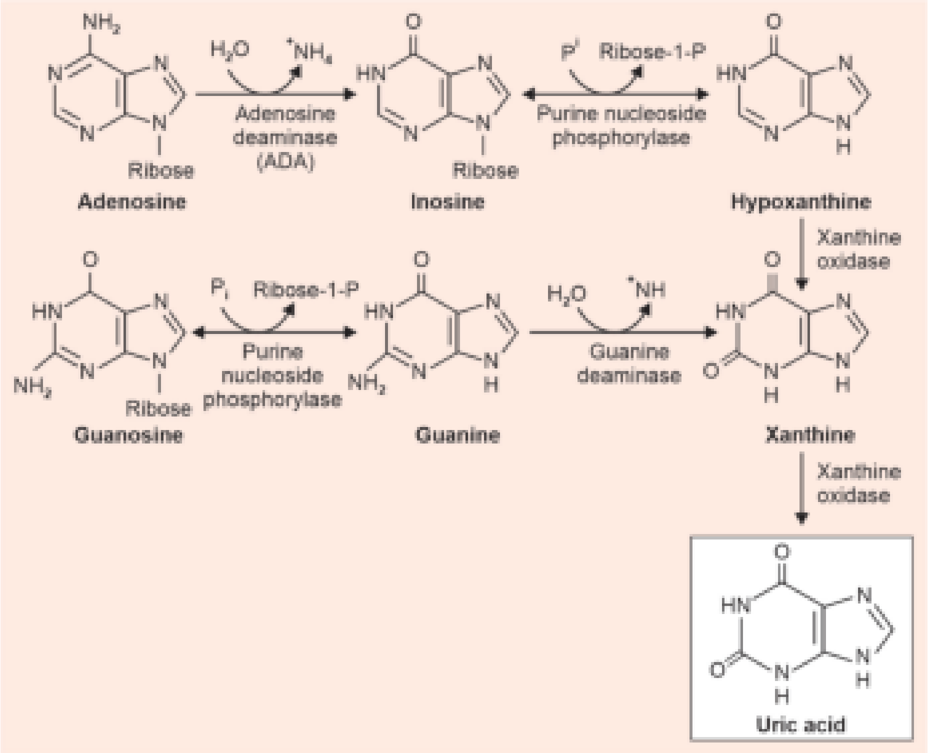

Adenosine
Ribose
H₂O
⁺NH₄
Adenosine deaminase (ADA)
Inosine
Pi
Ribose-1-P
Purine nucleoside phosphorylase
Hypoxanthine
Xanthine oxidase
Guanosine
Purine nucleoside phosphorylase
Guanine
Guanine deaminase
Xanthine
Uric acid

Fig. 7.11: Uric acid metabolism

excreted in urine, depends, to a large extent, on the purine content of the diet, and normally the excretion rate is 0.5–2.0 g/24 hours. The normal plasma uric acid level is 2.0–7.0 mg/dl.

URIC ACID EXCRETION

BAQ: What is uric acid? Write a brief note on uric acid excretion. What are the normal ranges of serum uric acid and 24 hours excretion of uric acid in urine?

Ans: The end product of purine catabolism in humans is uric acid. Uric acid is filtered in the glomeruli and partially reabsorbed by the tubules, and then it is excreted in the urine. The quantity excreted in urine, depends, to a large extent, on the purine content of the diet, and normally the excretion rate is 0.5–2.0 g/24 hours. The normal plasma uric acid level is 2.0–7.0 mg/dl. The uric acid concentration in erythrocytes is lower than in plasma. The normal range of uric acid in whole blood is 1–4 mg/dl. The plasma uric acid level is little affected by variations in the purine content of the diet and maintains a steady state between endogenous synthesis and urinary excretion.

Hyperuricemia and Hypouricemia

SAQ: Describe hyperuricemia and hypouricemia.

Ans: Hyperuricemia is most commonly defined by serum or plasma uric acid concentrations greater than 7.0 mg/dl in men or greater than 6.0 mg/dl in women. All the manifestations resulting from hyperuricemia are due to the low solubility of uric acid in water. Low serum uric acid values are observed in renal tubular defects, Fanconi syndrome, galactosemia, and heavy metal poisoning. Drugs such as aspirin (high doses), corticosteroids, allopurinol, and probenecid cause low serum uric acid levels.

Gout

LAQ: Write a note on gout.

Ans: Gout is associated with hyperuricemia. In gout, monosodium urate crystals precipitate from supersaturated body fluids. Gouty arthritis may be associated with urate crystals in joint fluid as well as with urate crystals in tissues surrounding the joint. The deposits may occur in other soft tissues also, and they elicit an intense inflammatory response consisting of polymorphonuclear leukocytes and macrophages. Increased excretion of uric acid may cause uric acid crystals to be deposited in the collecting tubules and lower urinary tract leading to calculi or stone formation with renal damage. Three types of kidney disease are caused by the precipitation of uric acid: Acute uric acid nephropathy, nephrolithiasis, and chronic urate nephropathy. Gout may be primary or secondary.

Primary gout: Most cases of this type are due to increased synthesis of purine nucleotides. It may be due to any one of the following reasons:

1. Abnormality in 5-phosphoribosyl aminotransferase activity, which is the rate-limiting enzyme of uric acid synthesis.
2. Abnormality in phosphoribosyl pyrophosphate (PRPP)), which is the ribose-5–phosphate donor for the *de novo* synthesis.
3. Deficiency of enzymes of salvage pathway: The recycling of purines formed by degradation of nucleotides *in vivo* is achieved by the salvage pathway. The key reactant in this pathway is PRPP. A deficiency of enzymes in this pathway results in increased availability of PRPP and decreased level of inhibitory purine nucleotides.

Secondary gout: It may be caused by reduced urinary excretion rate due to:

1. Renal failure
2. Lactic acid acidosis and ketoacidosis (lactic acid and ketoacids interfere with uric acid excretion).

3. Treatment with thiazide diuretics which inhibit tubular secretion of uric acid.
4. Hypertension and pre-eclamptic toxemia.

Secondary gout may also be caused due to increased turnover rate of nucleic acids. This may be observed in:

1. Rapidly growing malignant cells (and tissues), e.g. leukemias, lymphomas, polycythemia
2. Increased tissue breakdown after treatment of large malignant tumors.
3. Increased tissue damage due to trauma and raised rate of catabolism as in starvation.

SAQ: Mention two precipitating factors for an increase in serum uric acid.

Ans: Gouty attacks may be precipitated by a high purine diet (mainly non-vegetarian diet) and increased intake of alcohol. Increased intake of alcohol leads to the accumulation of lactic acid.

SAQ: What is the action of the drug allopurinol in treating high serum uric acid?

Ans: Allopurinol is a drug that inhibits the enzyme xanthine oxidoreductase and, thus, lowers the level of uric acid in the body. This may be useful in the treatment of gout, which is a disease caused by excess serum uric acid, forming crystals in joints.

Q: Describe gout under the following heads:

1. Biochemical basis
2. Clinical features
3. Diagnostic laboratory test
4. Treatment

Ans:

1. Biochemical basis: Gout is associated with hyperuricemia. It may be due to primary reasons such as abnormality in 5-phosphoribosyl aminotransferase activity, which is the rate-limiting enzyme of uric acid synthesis, abnormality in phosphoribosyl pyrophosphate (PRPP) or deficiency of enzymes of salvage pathway. Hyperuricemia also may be caused due to secondary reasons like, uncontrolled diabetes mellitus, renal disease, leukemia, polycythemia, etc.
2. Clinical features: In gout, monosodium urate crystals precipitate from supersaturated body fluids. Gouty arthritis may be associated with urate crystals in joint fluid as well as with urate crystals in tissues surrounding the joint. Acute severe pain in the metatarsophalangeal joint is the classic presentation of gout.

   The deposits of uric acid may occur in other soft tissues also, and they elicit an intense inflammatory response consisting of polymorphonuclear leukocytes and macrophages. Increased excretion of uric acid may cause uric acid crystals to be deposited in the collecting tubules and lower urinary tract leading to calculi or stone formation with renal damage.
3. Diagnostic laboratory tests: Gout can be diagnosed by serum uric acid determination when serum uric acid concentrations are greater than 7.0 mg/dl in men or greater than 6.0 mg/dl in women.
4. Treatment: Allopurinol is a drug that inhibits the enzyme xanthine oxidoreductase and, thus, lowers the level of uric acid in the body. This treatment is used for the management of primary conditions. However, for the treatment of a secondary condition, it is necessary to treat the secondary condition and accordingly if necessary, allopurinol is used.

Q: Describe Lesch-Nyhan syndrome under the following heads:

1. Biochemical basis
2. Clinical features
3. Diagnostic laboratory test
4. Treatment

Ans:

1. Biochemical basis: Lesch-Nyhan syndrome is a rare inborn error of purine metabolism characterized by the absence or deficiency

of the activity of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). Due to the deficiency of HPRT, the purines hypoxanthine and guanine are not used in the synthesis of nucleotides. The end product of hypoxanthine and guanine metabolism is uric acid. Hence very high levels of serum uric acid levels are observed in Lesch-Nyhan syndrome patients. In this clinical condition, sodium urate crystals may abnormally accumulate in the joints and kidneys.

2. Clinical features: The symptoms of Lesch-Nyhan syndrome include acute gouty arthritis, impaired kidney function, self-mutilating behaviors, and neurological impairment.
3. Diagnostic laboratory tests: Lesch-Nyhan syndrome can be diagnosed by serum uric acid determination when serum uric acid concentrations are greater than 7.0 mg/dl in men or greater than 6.0 mg/dl in women.
4. Treatment: Treatment is symptomatic. Hyperuricemia can be treated with allopurinol to control excessive amounts of uric acid.

   Allopurinol is a drug that inhibits the enzyme xanthine oxidoreductase and, thus, lowers the level of uric acid in the body.

PYRIMIDINE CATABOLISM

SAQ: Write a note on pyrimidine catabolism

Ans: Pyrimidines undergo ring cleavage, and the usual end products of catabolism are beta-amino acids in addition to ammonia and carbon dioxide. Pyrimidines from nucleic acids or the energy pool are acted upon by nucleotidases and pyrimidine nucleoside phosphorylase to yield the free bases. The 4-amino group of both cytosine and 5-methyl cytosine is released as ammonia. In the liver, ammonia is converted to urea and excreted in urine.

Multiple Choice Questions

Q1. When protein is metabolized, 90% of its nitrogen is excreted in the urine in the form of

A. Urea
B. Creatinine
C. Uric acid
D. Creatine

Q2. The normal nonprotein nitrogen level of blood is

A. 7–21 mg/dl
B. 25–50 mg/dl
C. 50–70 mg/dl
D. 0.7–1.5 mg/dl

Q3. The urea cycle consists of which of these reactions?

A. One mitochondrial and one cytosolic
B. Two mitochondrial and one cytosolic
C. Two mitochondrial and three cytosolic
D. One mitochondrial and two cytosolic

Q4. One turn of the urea cycle regenerates which molecule for another turn?

A. Citrulline
B. Arginine
C. Ornithine
D. Fumarate

Q5. Azotemia means, in blood, there is

A. High NPN
B. High creatinine
C. High urea
D. High uric acid

Q6. Urea nitrogen/creatinine ratio > 40 may be observed in

A. Prerenal condition
B. Renal condition
C. Postrenal condition
D. B and C

Q7. Which of these amino acids are involved in the synthesis of creatinine?

A. Glycine
B. Arginine
C. Methionine
D. A, B, and C

Q8. Presence of which of these molecules gives muscles a greater supply of energy than ATP?

A. GTP
B. ADP
C. Creatine phosphate
D. All of the above

Q9. Which is used by athletes and bodybuilders to gain muscle mass?

A. Creatine
B. Creatinine
C. Nucleic acids
D. NAD

Q10. Which one is not reabsorbed and secreted by the tubules?

A. Urea
B. Creatinine
C. Uric acid
D. A and C

Q11. Which of the following sugar is found in RNA?

A. 2-deoxyribose
B. 3-deoxyribose
C. D-ribose
D. D-xylulose

Q12. All are nucleosides *except*

A. Cytosine
B. Guanosine
C. Inosine
D. Adenosine

Q13. Serum urea nitrogen of a person with untreated diabetes mellitus was 35 mg/dl (Normal range: 7–21 mg/dl). High serum urea nitrogen in this case indicates

A. Renal condition
B. Pre-renal condition
C. Post-renal condition
D. A or C

Q14. Serum urea nitrogen of a person with untreated diabetes mellitus was 52 mg/dl (Normal range: 7–21 mg/dl) and serum creatinine was 3.8 mg/dl (Normal range: 0.7–1.7 mg/dl). High serum urea nitrogen and serum uric acid in this case indicate

A. Renal condition
B. Pre-renal condition
C. Post-renal condition
D. A or C

Q15. A 54-year-old obese female presented with generalized edema, loss of weight, and hypertension. What preferred laboratory tests are recommended to diagnose her clinical condition?

A. Serum urea nitrogen
B. Serum creatinine
C. Serum uric acid
D. Both A and B

Q16. A 48-year man presented with severe pain in his toes. What laboratory test is recommended to diagnose his clinical condition?

A. Serum urea nitrogen
B. Serum creatinine
C. Serum uric acid
D. Both A and B

Answers

1. A **2.** B **3.** C **4.** B **5.** A **6.** A **7.** D **8.** D **9.** A **10.** B

11. C **12.** A

13. B. Pre-renal condition. In uncontrolled diabetes mellitus, due to a deficiency of insulin, since glucose is not used by the cells of the body, amino acids are used excessively. The end product of amino acid metabolism is urea. Hence serum urea nitrogen was increased. There is no other laboratory value to indicate renal or post-renal condition.

14. D. Renal or post-renal condition. An increase in both serum urea nitrogen and serum creatinine indicates either renal or post-renal condition.

15. D. Both A and B. The symptoms point to renal disease.

16. C. Serum uric acid. High serum uric acid indicates gout. Acute severe pain in the metatarsophalangeal joint is the classic presentation of gout.

Case Studies

Case 1: A 33-year-old man experienced disturbances in urine volume (oliguria), signs of hypertension, and edema on his face and feet. Routine urine examination confirmed oliguria, proteinuria (+++), the presence of a large number of pus cells, epithelial cells, and casts in urine sediment. His laboratory test report values were as follows:

	Reference range (Normal range)
Blood urea nitrogen: 45 mg/dl	7–21 mg/dl

Serum creatinine: 2.3 mg/dl	0.6–1.2 mg/dl
Serum total proteins: 5.2 g/dl	6–8 g/dl
Serum albumin: 2.5 g/dl	3.3–4.8 g/dl

1. What is the probable diagnosis?

Ans: According to the laboratory test reports, excretory substances such as urea nitrogen and creatinine have increased in the blood (serum) and indicate that the kidneys of the patient are not able to excrete these substances in urine at a normal rate. Hence, is suffering from **kidney disease (renal condition).**

2. What is the mechanism behind the increase in serum urea nitrogen and creatinine?

Ans: The kidneys of this patient are not functioning normally; either due to microbial infection or autoimmune disease. Hence, serum urea nitrogen and creatinine have increased.

3. Why blood pressure of this patient was increased?

Ans: Since urine excretion was significantly decreased (oliguria), in response to that, due to increased secretion of renin, blood pressure was increased.

4. What was the reason for the edema seen on the patient's face?

Ans: Serum total protein and albumin were decreased significantly. Hence water from blood passed into the tissue spaces due to the osmotic effect.

5. What is the probable line of treatment?

Ans: It is necessary to find out the basic cause of renal disease and then according to the cause of the disease, treatment is suggested.

BAQ: Show horizontal integration of symptoms and test reports of case 1 with general medicine and pathology.

Ans: General medicine: Study of renal conditions.

Pathology: Study of cause of renal disease, pathophysiology and prognosis.

Case 2: A 45-year-old man complained of finger joint inflammation and severe pain. His laboratory test report was as follows:

	Reference range (Normal range)
Blood glucose (fasting): 87 mg/dl	70–110 mg/dl
Serum uric acid: 8.7 mg/dl	2–7 mg/dl
RA test: Negative	

1. What is the probable diagnosis?

Ans: The symptoms and increase in serum uric acid indicate that the patient is suffering from **primary gout**. He is not diabetic, and his RA (rheumatoid arthritis) test was negative, hence the possibility of secondary gout could be ruled out.

2. What is the mechanism behind the increase in serum uric acid?

Ans: Increase in phosphoribosyl pyrophosphate (PRPP) due to deficiency of enzymes that recycle PRPP in purine metabolism, leads to primary gout.

3. Why patient suffered from finger pain and inflammation of finger joints?

Ans: Increased uric acid crystals deposited in finger joints and irritate nerves that lead to finger pain and related inflammation.

4. What is the probable line of treatment?

Ans: Specific drug such as Allopurinol is prescribed, which inhibits the enzyme xanthine oxidoreductase and decreases the synthesis of uric acid.

BAQ: Show horizontal integration of symptoms and test reports of case 2 with general medicine and pathology.

Ans: General medicine: Study of gouty arthritis

Pathology: Study of cause of gout, pathophysiology and prognosis.

BAQ: Show vertical integration of symptoms and test reports of this patient with pharmacology.

Ans: Vertical integration with pharmacology

Study of appropriate drugs to treat high levels of serum uric acid.

Case 3: A 21-year-old young man sustained multiple injuries in a motorcycle accident. He was clinically dehydrated, and his blood pressure was 85/50 mmHg. His laboratory test reports were as follows:

	Reference range (Normal range)
Serum urea nitrogen: 48 mg/dl	7–21 mg/dl
Serum creatinine: 2.3 mg/dl	0.6–1.2 mg/dl

1. What is the probable diagnosis?

Ans: Excessive loss of blood in an accident, rise in serum urea nitrogen and serum creatinine indicate a **pre-renal condition**.

2. What is the mechanism behind the increase in serum urea nitrogen and serum creatinine?

Ans: The increase in serum urea nitrogen and creatinine is due to a decrease in the amount of blood circulating to kidneys, due to loss of blood in an accident. Hence, the glomerular filtration rate is decreased considerably leading to an increase in serum urea nitrogen and serum creatinine, although kidneys were normal.

3. Why blood pressure (BP) of this patient decreased?

Ans: Due to excessive loss of blood in the accident the amount of circulated blood to the kidneys and the heart was significantly low. Hence, decrease in BP.

4. What is the probable line of treatment?

Ans: Blood transfusion as soon as possible and thereafter normal saline drips, as the fluid replacement therapy.

BAQ: Show horizontal integration of symptoms and test reports of Case 3 with anatomy, physiology and nutrition.

Ans: Horizontal integration with anatomy

Insufficient blood supply to the kidneys

Horizontal integration with physiology

Decrease in glomerular filtration rate (GFR) and BP

BAQ: Show vertical integration of symptoms and test reports of this patient with pharmacology and transfusion medicine.

Ans: Vertical integration with pharmacology

Use of appropriate saline drips

Vertical integration with transfusion medicine

Testing of blood group, Rho (D), and appropriate blood transfusion.

Chapter

8

Water and Electrolyte Metabolism

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

Water is the most abundant compound in living cells, which contain 65–95% of water by weight in normal individuals. Water possesses several unique features due to its polarity and hydrogen bonding properties.

The electrolyte is a substance containing free ions that make the substance electrically conductive. **Electrolytes** are substances that are electrically charged.

Electrolytes are classified as either anions (negatively charged particles) that move toward an anode, or cations (positively charged particles) that move toward a cathode. Following are some examples of physiological electrolytes: Na^+, K^+, Cl^-, HCO_3^-, etc. and some organic anions such as lactate. Equal quantities of cations and anions are necessary to maintain homeostasis.

Competency achievement: The student should be able to:

BI6.9: Describe the functions of various minerals in the body, their metabolism, and homeostasis

WATER METABOLISM

SAQ: Enumerate functions of water in body metabolism.

Ans: Water is the most abundant compound in living cells, which contain 65–95% of water by weight in normal individuals. Water possesses the following several unique features due to its polarity and hydrogen bonding properties:

1. It is a powerful solvent for many ionic compounds and neutral molecules.
2. It has a strong influence, along with dilute salt solutions, on the state of dissociation of macromolecules in the cell.
3. It exerts a major influence on the structural and functional components of the cell.
4. The high heat of vaporization of water helps in body cooling by evaporation of moisture in the lungs and from the skin.

BAQ: Write a note on body fluid distribution.

Ans: Body fluid distribution:

Total body fluid is distributed between two main compartments: (A) Extracellular fluid and (B) Intracellular fluid.

A. Extracellular fluid: All of the fluid outside the body cells is collectively termed the extracellular fluid. It is heterogeneous and can be subdivided into (1) Plasma, (2) Interstitial and lymph fluid, (3) Dense connective tissue, cartilage, and bone, and (4) Transcellular fluids.

1. *Plasma:* It is the extracellular fluid of the blood.
2. Interstitial and lymph fluid may be considered to represent the actual fluid environment outside the cells. Continuous mixing and exchange of nutrients and metabolic waste products take place between plasma and interstitial fluid since they intermingle through pores of blood capillaries.
3. Dense connective tissue, cartilage, and bone do not exchange fluid or electrolytes readily with the remainder of the body water due to differences in structure and relative vascularity.
4. The transcellular fluids are the fluids found in the salivary glands, pancreas, liver, biliary tree, kidneys, gastrointestinal tracts, skin, gonads, thyroid gland, cerebrospinal fluid, and fluids within the spaces of the eye and the lumen of the gastrointestinal tract.

B. Intracellular fluid: The fluid within the body cells is called the intracellular fluid. The concept of a single intracellular fluid compartment is used since the fluid within each cell is fairly constant in composition.

BAQ: Write a note on intake and loss of body water.

Ans: Intake and loss of body water: The normal intake of water that forms in the body together averages 2500 ml/day. It can be approximately divided as follows for a day:

a. Intake of water and other fluids: 1200–1500 ml.
b. Foods: 770–1000 ml.
c. Metabolic water: 200–300 ml.

Water is lost from the body by the following four routes:

a. The skin: as sensible and insensible perspiration.
b. The lungs: As water vapor in the expired air.
c. The kidneys: As urine, and
d. The intestine: In the feces.

ELECTROLYTE METABOLISM

BAQ: Write the definition, classification, properties, and functions of electrolytes in the body's metabolisms.

Ans: Definition: Electrolytes are substances that are electrically charged.

Classification: Electrolytes are classified as either anions (negatively charged particles) that move toward an anode, or cations (positively charged particles) that move towards a cathode. Equal quantities of cations and anions are necessary to maintain homeostasis.

Following are physiological electrolytes: Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, HCO_3^-, $H_2PO_4^-$, HPO_4^{2-} and some organic anions such as lactate. Na^+, K^+, Mg^{2+}, and Ca^{2+} are the main cations, and Cl and HCO_3^- are the main anions present in the blood circulation.

Properties: The electrolytes in the body form various buffer systems. These buffer systems play a very important role in neutralizing excess acids and alkaline components formed during metabolic reactions. Maintenance of water balance between body fluids is another important function of electrolytes. Many cations are required for the activity of various enzymes. The electrolytes also play an important role in the regulation of nerve functions and muscular activities.

Functions: The maintenance of osmotic pressure and water distribution in the various body compartments is the primary function of four major electrolytes such as sodium, potassium, chlorides, and bicarbonate.

The electrolytes also play an important role in the maintenance of pH, proper heart and muscle functions, oxidation–reduction reactions, and as co-factors for enzymes. Almost all metabolic reactions are dependent on electrolytes.

BAQ: What are the factors that influence the distribution of body water?

Ans: Water is retained in the body in rather constant amounts. Its distribution is subjected to change by the osmotic forces which direct the movement of water from one compartment to another in the body. These osmotic forces are produced and controlled by the presence of the following substances in the body fluids:

1. Electrolytes: These are the most important substances which influence the distribution and retention of body water. Sodium (chief cation of extracellular fluid) and potassium (chief cation of intracellular fluids) are the most important osmotically effective electrolytes.
2. Organic substances of large molecular weight and size (serum proteins): An important function of the serum proteins is the maintenance of osmotic balance between the circulating blood and the tissue spaces. Albumin is of major importance in maintaining serum osmotic pressure. One gram of albumin holds 18 ml of fluid in the bloodstream. The serum proteins are amphoteric, and at the normal pH of the blood, they act as an acid and combine with cations (mainly sodium).
3. Organic compounds of small molecular size (glucose, urea, amino acids, etc.): These substances diffuse freely across cell membranes; hence they are not important in the distribution of water. They influence total body fluid, if present in large quantities.

MINERAL METABOLISM

Competency achievement: The student should be able to:

BI6.10: Enumerate and describe the disorders associated with mineral metabolism

LAQ: Write a note on electrolyte balance and dehydration. Define dehydration. How dehydration is diagnosed and treated?

Ans: Equal quantities of cations and anions are necessary to maintain homeostasis. Homeostasis is the state of maintenance of steady internal, physical, and chemeical conditions by living systems. Although amino acids and proteins also carry an electrical charge in solutions, their impact on electrolyte balance is considered insignificant.

The maintenance of osmotic pressure and water distribution in the various body compartments is the primary function of four major electrolytes such as sodium, potassium, chlorides, and bicarbonate. In addition to homeostasis, these electrolytes also play an important role in the maintenance of pH, proper heart and muscle functions, oxidation–reduction reactions, and as co-factors for enzymes. Almost all metabolic reactions are dependent on electrolytes.

The normal intake of water that forms in the body together averages 2500 ml/day.

Dehydration in the case of a person occurs when there is a loss of more water with electrolytes than the intake of water and electrolytes.

Water is lost from the body by the following four routes:

1. The skin: As sensible and insensible perspiration.
2. The lungs: As water vapor in the expired air.
3. The kidneys: as urine, and
4. The intestine: In the feces.

A 5–8% decrease in total body water can cause dizziness and fatigue. The loss of more than 10% of total body water can cause physical and mental deterioration and death may occur when loss of body water is more than 15% of the total body water, due to hypovolemic shock.

The following are the main reasons for dehydration and when there is no compensation for the loss of water and electrolytes:

1. Excessive loss of water from skin and lungs: Excessive sweating due to vigorous

activity and hot and humid weather increases the amount of sweat.

2. Severe diarrhea and vomiting mainly due to microbial infections lead to significant loss of water and electrolytes.
3. High fever may lead to dehydration.
4. Increase in the rate of urination due to hormonal imbalance. *Examples:* Due to a deficiency of insulin, the person may suffer from hyperglycemia leading to polyuria. Similarly, in ADH deficiency also person suffers from severe polyuria.
5. Certain medications, such as diuretics and some blood pressure medications, also may lead to dehydration due to the effects of the drugs.
6. When there is no access to safe drinking water during hiking, travelling, or camping.

Diagnosis of dehydration: The diagnosis of dehydration can be made from the following symptoms of dehydration of the patient: Dry mouth, skin and eyes, general discomfort, tiredness, thirst and nausea, headaches, loss of appetite, nausea, confusion, and seizures.

Treatment of dehydration: It is necessary to rehydrate the patient by giving fluids that contain electrolytes, such as electrolyte drinks, oral rehydration solutions, sports drinks, coconut water, or fresh fruit juices. In serious cases IV drips are necessary.

The rehydration solution can be prepared quickly using two cups of safe drinking water, 1/4th cup of lemon juice, and 1/8th tablespoon salt.

SODIUM METABOLISM

BAQ: Write a brief note on sodium metabolism.

Ans: The metabolism of sodium is influenced by adrenocortical steroids. Except for the androgens, all of the active corticosteroids increase the absorption of sodium and chloride by the renal tubules and decrease their excretion by the sweat gland, salivary gland, and gastrointestinal tract. Accompanying the retention of sodium by the kidney, there is increased excretion of potassium by an exchange of intracellular potassium with extracellular sodium.

BAQ: Write a brief note on potassium metabolism.

Ans: Metabolism of potassium is controlled by mineralocorticoids. The kidney is the principal organ of excretion for potassium. Potassium is not only filtered by the glomeruli but it is also secreted by the tubules. Variation in the extracellular potassium influences the activity of striated muscles so that paralysis of skeletal muscle and abnormalities in conduction and activity of cardiac muscle occurs.

SAQ: Write two important functions of sodium.

Ans:

1. Sodium is the major component of the cations of the extracellular fluid.
2. It is largely associated with chloride and bicarbonate in the regulation of acid–base balance.

SAQ: What is the normal range of serum sodium and the clinical significance of the determination of serum sodium?

Ans: The normal range of serum sodium is 133–148 mEq/L.

Clinical significance: Hyponatremia means low serum sodium levels and these are observed in the condition such as: (1) Severe prolonged diarrhea and vomiting, (2) salt losing nephritis and in (3) Addison's disease.

Hypernatremia means increased serum sodium values and these are observed in the conditions such as: (1) Severe dehydration, (2) diabetes insipidus (loss of dilute urine), (3) salt poisoning., (4) Cushing's syndrome and (5) In certain postrenal conditions (e.g. enlarged prostate) leading to obstruction to the flow of urine.

POTASSIUM METABOLISM

BAQ: What is the normal range of serum potassium and the clinical significance of the determination of serum potassium?

Ans: The normal range of serum potassium is 3.8–5.6 mEq/L.

Clinical significance: Hypokalemia means low potassium values and these are observed in the conditions such as: (1) Cushing's syndrome, (2) renal tubular damage, (3) metabolic alkalosis, and in (4) malnutrition.

Hyperkalemia means high potassium values and these are observed in the conditions such as: (1) Addison's disease, (2) Renal glomerular disease, and in (3) anuria and oliguria.

BAQ: Enumerate reasons for hyponatremia (sodium depletion) related symptoms and first-line treatment.

Ans: Hyponatremia (sodium depletion) occurs in:

1. Greatly diminished intake of sodium
2. General loss of both water and sodium, which is replaced by only water.
3. Burns, severe exudative skin lesions, and massive sweating.
4. Addison's disease (deficiency of mineralocorticoids)
5. Salt losing chronic nephritis. Diabetes ketoacidosis.
6. Loss in alimentary secretions due to prolonged vomiting and diarrhea.

When sodium is lost from the body, the extracellular fluid becomes hypotonic. Water leaves extracellular fluid to restore plasma osmotic pressure. More water is lost from tissue fluid. Symptoms begin to appear when the patient has lost the sodium equivalent of 4 liters of isotonic saline.

Symptoms: Vasoconstrictive shock, nausea, vomiting, cramps, intestinal dilation.

The cause of death is due to circulatory failure.

The first line of treatment: The treatment of choice is saline intravenously.

BAQ: Enumerate reasons for hypernatremia (sodium depletion) related symptoms and treatment.

Ans: Sodium excess (hypernatremia) occurs:

1. When there is an excessive intake of sodium chloride (mainly intravenously).
2. In Cushing's syndrome (excessive release of mineralocorticoids)
3. In head injury with water depletion.

Symptoms: There are raised central venous pressure, peripheral edema, and pulmonary edema with eventual respiratory failure.

The first line of treatment for hypernatremia: Infusion of hypotonic solutions such as 5% (W/V) dextrose in water, or if necessary, hemodialysis, to decrease serum sodium concentration.

BAQ: Write two important functions of potassium.

Ans:

1. Potassium is the principal cation of the intracellular fluid. Within the cells, it plays an important role in the maintenance of acid–base balance, osmotic pressure, and water retention. Intracellular potassium is essential for several important metabolic reactions catalyzed by enzymes.
2. It is also a very important constituent of the extracellular fluid because it influences muscle activity, notably the cardiac muscle.

BAQ: Enumerate reasons for hypokalemia (potassium depletion) related symptoms and the first line of treatment.

Ans: Potassium depletion is likely to develop in:

1. Gastrointestinal losses
2. Chronic wasting disease with malnutrition
3. Metabolic alkalosis

4. In prolonged intravenous administration of solutions that do not contain potassium
5. In Cushing's syndrome. In most of the above-mentioned cases, intracellular potassium is transferred to the extracellular fluid, and this potassium is quickly removed by the kidneys. A prolonged deficiency of potassium may produce severe damage to the kidneys. During heart failure, the potassium content of the myocardium becomes depleted.

Symptoms: The symptoms of hypokalemia include:

1. Muscle weakness with irritability and paralysis
2. Tachycardia and dilation of the heart with a change in the electrocardiogram.

The first line of treatment: Mild to moderate hypokalemia is typically treated with oral potassium supplements.

BAQ: Enumerate reasons for hyperkalemia (increase in potassium) related symptoms and the first line of treatment.

Toxic elevation of serum potassium is observed in the case of patients with: (a) Renal failure, (b) advanced dehydration, (c) shock, and in (d) Addison's disease.

Hyperkalemia may also occur if potassium is administered intravenously at an excessive rate.

Symptoms: The symptoms of hyperkalemia are mainly:

1. Cardiac and central nervous system depression
2. Mental confusion, weakness, numbness and
3. Weakness of respiratory muscles.

The first line of treatment: Administration of intravenous calcium gluconate.

Q: Write two important functions of chloride ions.

Ans:

1. As a component of sodium chloride, the element chlorine (as chloride ion) is essential in water balance, osmotic pressure regulation, and in acid–base balance.
2. In gastric juice, chloride also plays an important role in the production of hydrochloric acid.

Q: What is the normal range and clinical significance of serum chloride determination?

Ans: Normal range of serum chlorides: 95–106 mEq/L.

Low chloride values are observed in the conditions such as: (1) Prolonged vomiting, (2) burns, (3) salt losing renal disease, and (4) overhydration.

High chloride values are observed in the conditions such as: (1) Dehydration, (2) renal tubular disease, and in conditions causing decreased renal blood flow, i.e. congestive heart failure.

CSF ELECTROLYTES

BAQ: Enumerate normal values of cerebrospinal fluid (CSF), protein, glucose, sodium, potassium, and chlorides and the clinical significance of chemeical examination of these CSF components.

Ans: CSF	**Reference range (Normal values)**
Protein:	15 to 45 mg/dl
Glucose:	40–80 mg/dl
Chlorides:	700–750 mg/dl
Sodium:	144–154 mEq/L
Potassium:	2.0–3.5 mEq/L

Clinical significance: CSF glucose and chlorides significantly decrease in microbial infections and proteins increase, depending on the severity of the infection.

Competency achievement: The student should be able to:

PE13.11: Discuss the RDA, dietary sources of calcium, and its role in health and disease

CALCIUM METABOLISM

SAQ: Write two important functions of calcium.

Ans:

1. Calcium is present in the body in larger amounts than any other mineral element. It is mainly present in teeth and bone. About 99% of the body's calcium is in the skeleton. It is present in the bones as deposits of calcium phosphate in a soft, fibrous matrix.
2. It is also present in a small concentration of body fluids. The ionized calcium in the body fluids plays an important role in blood coagulation and in maintaining the normal excitability of the heart, muscles, and nerves.

BAQ: Write a note on bone calcium.

Ans: The mineral fraction of bone largely consists of hydroxyapatite type of crystal, $3Ca_3(PO_4)_2$. $Ca(OH)_2$, The other inorganic ions such as sodium, potassium, magnesium, carbonate, fluoride, and citrate are deposited on hydroxyapatite crystals. The organic matrix contains collagen type-1 (90%) and non-collageneous proteins such as osteocalcin. Organic matrix is mineralized by the deposition of inorganic calcium and phosphorus. The hypothetical two surfaces of the bone are: (A) Deposition surface and (B) Withdrawal surface.

A. Deposition surface is lined by osteoblasts. The functions of osteoblasts are:
   1. Manufacturing of bone matrix consisting of collagen, mucopolysaccharides, and osteocytes.
   2. They secrete alkaline phosphatase. It is presumed that the alkaline phosphatase causes the liberation of phosphate ions which increase calcium phosphate product, and then it is deposited in the matrix.

B. The withdrawal surface of the bone is lined by osteoclasts. Reabsorption of bone occurs by the demolition of matrix and minerals by the osteoclasts and osteocytes. This process takes place with a low plasma concentration of calcium and phosphate.

The mineral deposits of bone are in a dynamic state, constantly being formed and reabsorbed. It is more rapid during childhood and at a slower declining rate during adult life. Continuous turnover (remodeling) of bone helps to repair damage and adjust strength. The remodeling cycle can be divided into the following processes: Activation, reabsorption, reversal, formation, and resting phase.

Bone growth and turnover are influenced by the metabolism of calcium, phosphorus, magnesium, and main hormones such as parathyroid hormone, and thyroid hormone, and also by other androgens, estrogens, cortisol, insulin, and various growth hormone factors.

SAQ: Enumerate the factors that maintain the concentration of calcium, phosphate, and magnesium in plasma.

Ans: The concentration of calcium, phosphate, and magnesium in plasma is dependent on the net effect of: 1. Bone mineral deposition, and reabsorption, 2. intestinal absorption, and 3. renal excretion.

SAQ: What is the average calcium of the human body, skeleton, soft tissue, and extracellular fluid?

Ans: An average human body contains approximately one kilogram of calcium. The skeleton contains 99% of the body's calcium, and soft tissues and extracellular fluids contain about 1% of the body's calcium.

BAQ: Write a short note on body calcium.

Ans: An average human body contains approximately one kilogram of calcium. The skeleton contains 99% of the body's calcium, and soft tissues and extracellular fluids contain about 1% of the body's calcium.

Physiologically, calcium is classified as either intracellular or extracellular. The skeleton is a major reservoir for providing both the intracellular and extracellular pools. The concentration of intracellular calcium is usually 1/1000 of extracellular calcium, but intracellular calcium has very important roles in many physiological functions, such as muscle contraction, hormone secretions, glycogen metabolism and cell division.

SAQ: What are the sources of calcium and normal average daily intake of calcium?

Ans: Milk and cheese are the richest sources of calcium. Other foods such as egg yolk, beans, lentils, nuts, figs, and cabbage also contain calcium. The normal daily intake of calcium in adults is 0.5–1.0 g.

SAQ: Write a note on the absorption of calcium. What are the factors that influence the absorption of calcium?

Ans: Absorption of calcium in the small intestine is facilitated by vitamin D, lactose, and a high-protein diet. About 15% of the dietary calcium is absorbed. About 200 mg of calcium is excreted in urine per 24 hours, and the remainder is recovered in feces.

The factors which influence calcium absorption in the intestine are:

1. Phytic acid in cereal grains forms insoluble calcium phytate.
2. Oxalates form calcium oxalates.
3. pH: The more alkaline the intestinal contents, the less soluble the calcium salts. Acidic pH promotes calcium absorption.
4. Phosphate: If Ca: P ratio is high, much $Ca_3(PO_4)_2$, will be formed, and absorption is diminished.
5. Presence of free fatty acids: Due to impaired fat absorption, fatty acids may react with calcium to form insoluble calcium soaps.
6. Presence of vitamin D: It promotes absorption of calcium in the intestine.

LAQ: Write a note on the absorption and metabolism of calcium.

Ans: Calcium present in food is absorbed across the brush border membrane of intestinal epithelial cells. After the cellular uptake, calcium is immediately bound to a vitamin D-dependent calcium-binding protein, which transfers the calcium directly into the endoplasmic reticulum of epithelial cells. From there calcium is actively transported into the body cells. Active transport of calcium occurs primarily in the duodenum. The active absorption of calcium from the gut is regulated by calcitriol.

After the absorption of calcium and phosphorus, the metabolism of these ions is controlled by the following hormones.

1. Parathyroid hormone: It is a hormone of a single active polypeptide. It is secreted by the parathyroid gland. The accepted actions of the parathyroid hormone are as follows:
   i. Activation of osteoclasts and osteocytes to promote dissolution of the matrix, breakdown of bone salts, and liberation of calcium and phosphate.
   ii. Increases phosphate excretion by diminishing tubular reabsorption of phosphorus. Increases reabsorption of calcium.
2. Calcitonin: Calcitonin is secreted by the C cells of the thyroid gland. It is a calcium- and phosphate-lowering hormone. Calcitonin inhibits osteoclast function and decreases renal resorption of calcium and phosphate.
3. Other hormones: Thyroxin stimulates osteoclasts activity.

   Estrogen and androgens influence bone metabolism by promoting the production of bone matrix.

Glucocorticoids, by their action on protein catabolism, affect the production of bone matrix.

The action of vitamin D: Another factor that influences calcium and phosphorus metabolism is vitamin D (calciferol) which acts in the intestine. It promotes the absorption of both calcium and phosphorus in the small intestine. It also acts directly on the bone to promote normal growth, development, and utilization of calcium.

The calcium in the blood is confined to the serum. Normal total serum calcium ranges from about 9 to 11 mg/dl. The ionized calcium ranges from 5.9 to 6.5 mg/dl. Non-ionized diffusible calcium ranges from 0.15 to 0.51 mg/dl and the non-diffusible protein-bound calcium ranges from 3.5 to 4.0 mg/dl. When blood passes through the kidneys, filtered calcium is reabsorbed by the proximal part of the tubules and this absorption is under the influence of parathyroid hormone and vitamin D.

Excretion of excess calcium takes place through the bile and feces. Urinary excretion of calcium is normally about 200 mg per day. Normal serum calcium levels are maintained by the appropriate actions of parathyroid secretions and vitamin D.

LAQ: Write a note on skeletal homeostasis.

Ans: Skeletal homeostasis is dependent on the specific balance between bone formation and bone resorption. Osteoblasts are located along the bone-forming surface. These are responsible for laying down the osteoid seam (unmineralized matrix), which is calcified after about 10 days. Osteoclasts form multinucleated giant cells in lacunar spaces and are responsible for bone resorption.

Eighty percent of the skeletal mass is made up of cortical bone, which is a compact structure of dense bone. About 20% of trabecular bone consists of a delicate interlacing of dense bone with bone marrow. Although cortical bone makes up the majority of the skeletal mass, its surface area and, the resorptive surface are much less than that of trabecular bone.

The major protein component of bone is collagen. It consists of a triple-helical conformation. There are two identical alpha-1 and alpha-2 chains (collagen type I) found specifically in skin and bone. About 10% of the organic bone matrix consists of non-collagen components. These include osteocalcin, osteonectin, osteopontin, acid mucopolysaccharides, and lipids. The inorganic component of bone is primarily composed of calcium and phosphate in the form of hydroxyapatite crystals.

Bone remodeling is a continuous process consisting of bone formation, mineralization, and bone resorption. Insulin, insulin-like growth factors I and II, androgens, estrogens, growth hormone, and thyroid hormone increase the rate of bone formation. Calcitonin, phosphates, and diphosphonates inhibit bone resorption. Parathyroid hormone and 1, $25(OH)_2$ D_3 increase osteoclastic activity and subsequent bone resorption and release calcium into the circulation. Glucocorticoids decrease the rate of bone formation and increase the rate of bone resorption, which leads to a net loss of bone. In hyperthyroidism, loss of calcium occurs since the resorption rate exceeds the rate of bone formation.

Calcium plays an important role in skeletal homeostasis. It acts as an intracellular signaling ion involving the plasma membrane, and blood coagulation mechanisms require calcium ions.

BAQ: What are the factors that affect serum calcium?

Ans: The level of serum calcium may be affected by:

1. Deficient calcium absorption from the intestine (steatorrhea).
2. Alterations in the amount of parathyroid hormone secreted: Reduced serum calcium: Hypoparathyroidism. Increased serum calcium: Hyperparathyroidism.
3. Changes in serum inorganic phosphorus (renal failure) and
4. Alterations in plasma proteins (proteinuria).

   In the case of points 1, 2, and 3, the physiologically active (ionized) calcium is affected, and in the case of point 4, the physiologically inactive (protein-bound) calcium is affected. A reduction in serum calcium affecting the ionized calcium is one of the causes of tetany.

5. *Hypervitaminosis D:* Excessive dosage of vitamin D may raise serum calcium.
6. *Vitamin D deficiency*: It may lead to rickets, a clinical condition in which serum calcium is most often either just within normal limits or slightly below the normal serum level. Low serum calcium is frequently observed in adult rickets (osteomalacia).

SAQ: What is the normal range and clinical significance of serum calcium determination?

Ans: Normal range of serum calcium is 9.0-11.0 mg/dl.

Clinical significance: Decreased serum calcium values are found in hypoparathyroidism, rickets, osteomalacia and steatorrhea. A fall in serum calcium can occur in acute pancreatitis and in those forms of renal diseases in which excessive proteinuria is observed.

Increased serum calcium values are observed in hyperparathyroidism, hypervitaminosis D and multiple myeloma.

Competency achievement: The student should be able to:

PE13.12: Describe the causes, clinical features diagnosis and management of calcium deficiency

BAQ: Describe osteoporosis under the following heads:

1. Biochemeical basis
2. Clinical features
3. Diagnosis
4. Treatment

Ans:

1. **Biochemeical basis:** Osteoporosis is defined as a reduction of bone mass. This makes patients more susceptible to fractures.

   Postmenopausal women suffer from estrogen deficiency. Postmenopausal women are susceptible to primary osteoporosis since osteoporosis is closely related to estrogen deficiency. During the menopausal transition period, the drop of estrogen leads to more bone resorption than formation, resulting in osteoporosis.

   Osteoporosis in men is associated with a decline in gonadal function with aging, delayed puberty, hypogonadotropic hypogonadism, and alcoholism.
2. **Clinical features:** The imbalance between osteoblastic and osteoclastic activity in menopause results in thinning of the cortical bone, resorption of the cancellous bone spicules, enlargement of the medullary cavity, and the formation of narrow, delicate trabeculae.
3. **Diagnosis:** Serum calcium, inorganic phosphorus, and vitamin D determination are useful in the diagnosis of osteoporosis.
4. **Treatment:** The treatment for osteoporosis is by prescribing bisphosphonates like alendronate, ibandronate, etc. Nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate synthase, which is necessary for promoting the attachment of the osteoclast to the bone. By the action of the drug, the osteoclast detaches from the bone surface, thus inhibiting bone resorption.

BAQ: Write a short note on osteoporosis.

Ans: Osteoporosis is defined as a reduction of bone mass. This makes patients more susceptible to fractures. Postmenopausal women suffer from estrogen deficiency. Postmenopausal women are susceptible to primary osteoporosis since osteoporosis is closely related to estrogen deficiency. In osteoporosis, the imbalance between osteoblastic and osteoclastic activity results in thinning of the cortical bone, resorption of the cancellous bone spicules, enlargement of the medullary cavity and the formation of narrow, delicate trabeculae. The mineralization of bone is normal in osteoporosis, but the bone mass is decreased. The risk of osteoporosis and fracture is related to the bone mass at menopause and the rate of bone loss after menopause. In postmenopausal bone loss (osteoporosis type I) excessive osteoclastic activity affects primarily trabecular bone and vertebral fractures are more common. However, senile or aging-associated bone loss (osteoporosis type II) is associated with a decline in osteoblastic activity and an increased frequency of hip fractures of cortical bone. The combined use of bone mineral density (BMD) at menopause (to estimate bone mass) and biochemeical markers (to estimate bone loss rate) is the best predictor of future fracture rate. Biochemeical markers are separated into markers of bone formation and markers of bone resorption.

Osteoporosis in men is associated with a decline in gonadal function with aging, delayed puberty, hypogonadotropic hypogonadism and alcoholism.

Hypercortisolism causes a profound decrease in bone mineral density (BMD) through inhibition of osteoblast function and a subsequent decrease in bone formation, as indicated by a decrease in the osteoid seams and a low mineral apposition rate.

BAQ: Write a short note on Paget's disease.

Ans: Paget's disease is a chronic skeletal disorder that affects 3 to 4% of both men and women over the age of 50 years. The cause of this disease is unknown. In Paget's disease, increased bone resorptive activity and an increased number of abnormally enlarged osteoclasts are observed. These osteoclasts have greatly increased the number of nuclei coupled with the ability of rapid osteoblast-mediated bone formation. This results in architecturally inferior bone formation. The abnormal bone and its increased tendency to fracture can cause compression of neural structure. Laboratory tests related to bone profile tests and mainly increased values of serum alkaline phosphatase indicate an increase in bone turnover and markers of bone formation and bone resorption.

Treatment of Paget's disease is focused on providing physical assistance, which includes the addition of wedges in the shoe as walking aids and supportive physiotherapy.

The treatment of osteoporosis is by prescribing bisphosphonates like alendronate, ibandronate, etc. By the action of the drug, the osteoclast detaches from the bone surface, thus inhibiting bone resorption.

PHOSPHORUS METABOLISM

SAQ: Write the importance of phosphorus.

Ans: About 80% of the total phosphorus is combined with calcium in bones and teeth. It is found in every cell of the body. About 10% is combined with proteins, lipids and carbohydrates, and other compounds in blood and muscle. The remaining 10% is widely distributed in various chemeical compounds such as phospholipids, nucleotides, nucleosides, DNA, RNA, etc.

SAQ: What are the daily requirements and sources of phosphorus?

Ans: The daily intake of phosphorus in adults is 1.5–3.0 g.

The two main sources of dietary phosphorus are organic phosphorus and inorganic phosphorus. Organic phosphorus is present in animal and vegetarian proteins, and inorganic phosphorus is mostly in processed food. Animal-based foods and plants are abundant in organic phosphorus.

SAQ: Write a note on the absorption of phosphorus.

Ans: About 40% to 60% of animal-based phosphorus is absorbed. Vitamin D is required for the absorption of phosphorus. Plant phosphorus, mostly associated with phytates, is poorly absorbed by the human gastrointestinal tract. Inorganic phosphorus in processed foods such as cheese and soda drinks is absorbed well. Inorganic phosphate is absorbed as the phosphate ion. Phospholipids or nucleic acid phosphates are liberated by enzymatic hydrolysis. When there is defective absorption of calcium, defective absorption of phosphorus usually results.

BAQ: Write a note on the metabolism of phosphorus.

Ans: Dietary phosphorus, in inorganic and organic forms, is digested in the upper gastrointestinal tract. Absorbed phosphorus is transported to bone, skeletal muscle, soft tissues, and kidney, and exchanged at the rate determined by concentration, rate of blood flow, and activity of inorganic phosphorus transporters. During growth, there is net utilization of phosphorus, and with advancing age net loss of phosphorus takes place. The bone phosphorus reservoir is depleted and repleted according to phosphorus requirements. Extracellular inorganic phosphorus is maintained by the kidney tubular reabsorption under the control of parathyroid hormones.

SAQ: What is the normal range and clinical significance of serum inorganic phosphorus determination?

Ans: Normal range of inorganic phosphorus in adults is 2.5–4.5 mg/dl. The normal range of inorganic phosphorus in children is 4.0–7.0 mg/dl.

Clinical significance: Decreased serum phosphorus values are observed in preliminary hyperparathyroidism, rickets (vitamin D deficiency), and Fanconi's syndrome (a disease associated with a defect in the reabsorption of phosphorus). Increased serum phosphorus levels may be found in hypervitaminosis D, hypoparathyroidism, and renal failure.

BAQ: Write a note on rickets and osteomalacia.

Ans: These disorders are caused by various clinical conditions associated with vitamin D deficiency or resistance. Rickets is observed in children. In children, the growing skeleton is involved. The term osteomalacia is used for the mineralization disorder in adults. The characteristic feature of these disorders is an increase in osteoid volume and thickness and a decrease in calcification of the mineralization. Poorly mineralized long bones are incapable of withstanding stress and tend to undergo deformity such as the bowing of legs.

A deficiency of vitamin D leads to insufficient absorption of calcium and inorganic phosphorus in the intestine, leading to secondary hyperparathyroidism. In hyperparathyroidism, excessive calcium is released from the bones. Decreased serum inorganic phosphorus with low-normal calcium is a significant observation in rickets and osteomalacia. Clinical manifestations in these conditions are skeletal deformities, susceptibility to fracture, hypotonia, and weakness. Children are unable to walk without support. Serum alkaline phosphatase levels are usually increased in rickets and osteomalacia.

Rickets

LAQ: Write a note on rickets, signs and symptoms, common causes, diagnosis, treatment, and preventive measures.

Ans: Rickets is a condition that results in soft and weak bones in children. The following are important features of rickets: Bowed legs, stunted growth, bone pain, large forehead, and disturbed sleep. Genetic causes of rickets (hereditary rickets) are rare. These account for about 13% of total rickets.

Signs and symptoms of rickets: Signs and symptoms of rickets include bone tenderness and susceptibility to bone fractures, particularly greenstick fractures. A greenstick fracture is a fracture in a young, soft bone in which the bone bends and breaks.

Early skeletal deformities can arise in infants such as soft, thinned skull bones (craniotabes), which is the first sign of rickets. Skull bossing (protuberance of the skull) may be present as a delayed closure of the fontanelles.

Young children may have bowed legs and thickened ankles and wrists. Older children may have knocked knees. Spinal curvatures (kyphoscoliosis) or lumbar lordosis may be present. The pelvic bones may be deformed.

The common causes of rickets: The most common cause of rickets is vitamin D deficiency. Vitamin D deficiency may result from a diet deficient in vitamin D, insufficient sun exposure, exclusive breastfeeding without vitamin D supplementation, celiac disease, and certain genetic conditions. Other factors may include a diet containing insufficient calcium or phosphorus. The underlying mechanism involves insufficient calcification of the growth plate.

Maternal deficiencies may be the cause of overt bone disease before birth and impairment of bone quality after birth. The primary cause of congenital rickets is vitamin D deficiency in the mother's blood, which the baby shares.

Congenital rickets may also be caused by other maternal diseases, including severe osteomalacia, untreated celiac disease, malabsorption, pre-eclampsia, and premature birth. Rickets in children is similar to osteoporosis in the elderly, with brittle bones. Pre-natal care includes checking vitamin levels and ensuring that any deficiencies are supplemented.

Diagnosis of rickets: Diagnosis is generally based on blood tests finding low calcium, low phosphorus, and a high alkaline phosphatase together with X-rays report of the bones.

Preventive measures: Prevention of rickets includes vitamin D supplements for exclusively breastfed babies.

Treatment: Treatment depends on the underlying cause. If it is due to a lack of vitamin D, treatment is usually with vitamin D and calcium. This usually results in improvements in the blood levels of vitamin D and calcium within a few weeks. Bone deformities may also improve over time. Occasionally surgery may be done to fix bone deformities. Genetic forms of the disease require specialized life-long treatment.

BAQ: Write a note on the treatment and management of rickets.

Ans: Treatment of rickets is usually with vitamin D and calcium. This usually results in improvements in the blood levels of vitamin D and calcium within a few weeks.

The American Academy of Pediatrics (AAP) recommends an initial 2- to 3-month regimen of "high-dose" vitamin D therapy of 1000 units daily in neonates, 1000 to 5000 units daily in infants 1 to 12 months old, and 5000 units daily in patients over 12 months old.

Supplementation: Sufficient vitamin D levels can also be achieved through dietary supplementation (about 500 ml, vitamin D-fortified milk or formula) per day and exposure to sunlight (minimum 30 minutes).

The diet recommended foods that contain vitamin D such as butter, eggs, fish liver oils, margarine, fortified milk, juice, mushrooms, mutton, chicken and fish.

Although radiologic evidence of healing occurs within 2 to 4 weeks of treatment, large dose treatment (of either vitamin D_3 or D_2) should be continued for 2 to 3 months. After sufficient calcitriol concentrations are achieved, a maintenance dose of 400 units of vitamin D daily is recommended in all age groups. Larger maintenance doses (800 units per day) may be considered in the following at-risk populations: Premature infants, dark-skinned infants and children who reside in areas of limited sun exposure (>37.5° latitude), obese patients (due to fat sequestration of vitamin D), and those on following medications known to compromise vitamin D: Antibiotics—rifampin (rifampicin) and isoniazid, commonly used to treat TB, anti-seizure drugs—phenobarbital, carbamazepine, phenytoin and anti-cancer drugs—Taxol and related compounds.

BAQ: Write a note on the management of genetic rickets.

Ans: A lifelong treatment is required of phosphate and calcitriol replacement to restore bone mineralization and improve skeletal deformities for a patient suffering from genetic rickets.

Calcitriol is recommended at doses ranging from 25 to 70 ng/kg/day (2 doses) with phosphate salts (sodium phosphate, potassium phosphate). It can be given in tablet or solution form both of which are equally effective.

Patients should be monitored for clinical, anthropometric, and laboratory characteristics at three-month intervals.

Laboratory assessments include serum calcium, phosphate, serum alkaline phosphatase, and parathyroid hormone levels, as well as urinary calcium and creatinine for hypercalciuria.

In addition, renal ultrasonography should be performed annually, before and after treatment, to monitor the development of nephrocalcinosis. Skeletal X-ray is recommended to be performed annually before treatment and during treatment for monitoring of skeletal findings.

BAQ: Enumerate high-risk factors for developing rickets:

Ans: Those at higher risk for developing rickets include:

1. Breastfed infants whose mothers had low levels of serum calcium, and vitamin D and are not exposed to sunlight.
2. Breastfed infants who are not exposed to sunlight.
3. Adolescents, in particular when undergoing the pubertal growth spurt.
4. Any child whose diet does not contain enough vitamin D or calcium.
5. Diseases causing soft bones in infants, like hypophosphatasia
6. Use of specific face and body creams that block exposure to ultraviolet light.
7. Hoods and veils act as sunlight barriers that prevent individuals from synthesizing vitamin D naturally from the sun.

IRON METABOLISM

Competency achievement: The student should be able to:

PE13.1: Describe the RDA, dietary sources of iron, and their role in health and disease

LAQ: Write a note on the importance of iron, sources, RDA, causes of iron deficiency, iron deficiency symptoms, diagnosis, and treatment.

Ans: Iron is the most important component of hemoglobin, a compound protein in red blood cells that carries oxygen from the lungs to all parts of the body. Iron is an important part of the protein myoglobin, that carries and stores oxygen in muscle tissues. Iron also plays an important role in the normal development of the brain and growth in children. Iron is required for the normal production and function of various cells and hormones.

The recommended dietary allowance (RDA):

Men: 8 mg/day.

Females: 18 mg/day

Post-menopausal women: 8 mg/day.

Children: 8 mg/day

Iron deficiency: Iron deficiency leads to iron deficiency anemia (IDA). IDA may develop into the following two stages: (1) The progressive depletion and ultimate exhaustion of available tissue iron stores and (2) the development of anemia.

The following are three major factors in the pathogenesis of IDA:

1. In increased physiological demand for iron.
2. Pathological blood loss, and
3. Inadequate iron intake

Increased physiological demand for iron: During the period of growth, there is a progressive increase in the number of red cells in the body and consequently in the total amount of hemoglobin. This results in an increased demand for iron.

During the reproductive life of the female, menstruation, pregnancy, parturition, and lactation significantly increase the physiological requirements for iron. Each pregnancy requires about 500–600 mg for the fetus to cover blood loss at parturition.

Pathological blood loss: Blood may be lost through the gastrointestinal tract or urinary tract due to microbial and worm infections, trauma, ulcers, or cancerous growth. Loss of blood to any extent causes a lowering of the total body iron since 60–70 percent of the total iron content of the body is confined (contained) in the hemoglobin of red cells. The normal adult has tissue iron reserves sufficient to replace between one-third and one-half of the circulating hemoglobin. Once this reserve is exhausted, continued bleeding causes a state of iron deficiency.

Inadequate intake: Inadequate intake may result from either nutritional deficiency or impaired absorption. Nutritional deficiency may be present as a result of an inadequate diet in infants and young children. It may occur in adults due to poor economic circumstances, specific dietary habits or dislikes, and anorexia (especially in pregnancy).

A long-standing impairment of iron absorption: A long-standing impairment of iron absorption may result from gastrectomy or gastroenterostomy, tropical sprue, or coeliac disease in either children or adults.

Important serum components that indicate iron deficiency: Serum ferritin represents a storage form for iron. The concentration of ferritin in adults ranges between 15–300 µg/L and mean levels in men and women are 123 µg/L and 56 µg/L respectively. In iron deficiency, concentrations of ferritin are less than 12 µg/L.

Iron deficiency anemia (IDA) signs and symptoms: Iron deficiency anemia signs and symptoms: Weakness, extreme fatigue, headache, chest pain, dizziness, pale skin, cold hands and feet, brittle nails, and loss of appetite.

Diagnosis of iron deficiency anemia (IDA): Complete blood count determination and iron using EDTA-blood are used for the diagnosis of IDA.

In adult males and females normal range of iron is 60–150 µg/dl. The normal range of hemoglobin in men is 14–16 g/dl and the normal range of hemoglobin in females is 13–15 g/dl.

Significant decreases in hemoglobin, ferritin, and iron, microcytic and hypochromic red cells are the main observations in IDA.

Dietary sources of iron: Iron is present in foods such as meat, egg yolk, fish, nuts, legumes, dates, etc. About 10 to 20 mg of iron is consumed per day by an average individual. Milk is, however, deficient in iron.

Treatment: It is necessary to find out the primary cause of iron deficiency and treatment is given accordingly. Once the primary cause is diagnosed and treated, care is taken to provide sufficient iron with a diet rich in iron as mentioned above. Oral therapy includes iron in tablets or syrup in the form of hemoglobin preparations, and 10 ml BD after meals up to six months. It is necessary to determine complete blood count (CBC) after every two months to follow the effects of the therapy.

LAQ: Write a note on iron absorption.

Ans: Food iron can be divided into two types: Non-heme iron and heme iron. The absorption of non-heme iron is facilitated by ascorbic acid and retarded by phytic acid and phosphates, whereas heme iron does not depend on their presence.

Iron is absorbed from the upper part of the small intestine, including the duodenum.

Although iron absorption does not occur in the stomach, the hydrochloric acid present in the stomach renders the iron of the food soluble and then facilitates its absorption. Most food iron is in a ferric state and ascorbic acid converts the ferric iron into ferrous iron in the gastrointestinal tract. Iron absorption occurs when the iron is in a ferrous state.

Absorption of iron is most efficient in the duodenum and proximal jejunum. Non-heme iron attaches to surface glycoprotein receptors on the brush border of the mucosal absorptive cells. Depending upon the requirement of the body, a proportion (bound to transferrin) is rapidly transferred into the portal circulation and distributed to the tissue iron stores. Most of the remaining iron in the mucosal cell combines with apoferritin to form ferritin. Heme enters the mucosal cell unchanged, and the iron is released within the cell by the action of the enzyme, heme oxygenase. Iron from this source then enters the common iron pool of the mucosal cell. Apart from the amount of available iron in the diet, the main factors which influence the amount of iron absorption are: (1) The size of the iron stores and (2) the rate of erythropoiesis.

After absorption, iron is bound with transferrin (β-globulin of plasma protein). Iron which is not utilized for the synthesis of hemoglobin combines with apoferritin (an apoprotein) and the complex formed is ferritin, which is stored in the liver. The stored iron is recycled to form heme and other compounds. In a normal individual, about 4 g of iron is present. About 70% of it is present in hemoglobin, 20% in ferritin and the rest of the iron is present in the various compounds such as myoglobin, cytochromes, cytochrome oxidase, peroxidase, etc. present in tissues.

SAQ: What are the dietary sources of iron?

Ans: The chief dietary sources of iron are liver, kidney, egg yolk, and green vegetables.

SAQ: What is the daily normal intake and absorption of iron in adults?

Ans: The daily intake of a normal adult contains 10–20 mg of iron. Absorption of iron is about 10% and it is greater in women than in men.

SAQ: Enumerate the factors that enhance iron absorption.

Ans: The following factors enhance iron absorption: Meat, fish, poultry, seafood, gastric acid, ascorbic acid and citric acid.

SAQ: Enumerate the dietary factors that inhibit iron absorption.

Ans: The following factors inhibit iron absorption: Phosphates, calcium, tea (tannic acid), coffee, colas, soy protein, high doses of minerals, and bran.

SAQ: Enumerate the conditions that lead to the loss of iron from the body.

Ans: Heavy and lengthy menstrual periods, rapid growth, pregnancy (recent or current), inflammatory bowel disease, frequent blood donations, parasitic infection, chronic use of aspirin or nonsteroidal anti-inflammatory drugs (e.g. ibuprofen) or corticosteroid use leading to ulcers in the gastrointestinal tract.

LAQ: Write a note on the transportation of iron in the body.

Ans: After the absorption in the small intestine, about 3–4 mg of iron circulates in serum bound to transferrin (a beta-globulin synthesized in the liver). Each molecule of transferrin binds one or two atoms of ferric iron. The function of transferrin is to transport iron absorbed from the alimentary tract to the tissue stores, from tissue storage to bone marrow erythroblasts, and from one storage site to another storage site. Transferrin receptors are present in reticulocytes and erythroblasts.

When transferrin reaches the storage sites or the bone marrow, it attaches to specific receptors on cells, and the bound transferrin is internalized within the cell before the release of iron. The liberated ferric ions pass into the cell for storage or utilization. The total amount of transferrin in the plasma is about 8 g and a similar amount is present in the extracellular fluid in equilibrium with plasma transferrin.

The level of serum iron in normal individuals averages about 20 μmol/L. Transferrin is present in serum in a concentration that enables it to combine with 44–80 μmol/L of iron per liter. This value is known as the total iron-binding capacity of the serum. The percentage of the total iron-binding protein to which iron is attached is known as the percentage saturation of the iron-binding protein. This is calculated by dividing the serum iron value by the total iron-binding capacity and the results are expressed as a percentage. The average normal value is about 33 percent saturation (i.e. the iron-binding protein is about one-third saturated).

The concentration of ferritin is related to body iron stores, and it is age and sex-dependent. Serum ferritin concentrations in adults range between 15–300 μg/L and mean levels in men and women are 123 μg/L and 56 μg/L respectively. In iron deficiency, concentrations of ferritin are less than 12 μg/L. In iron overload, levels of ferritin are very high. Serum ferritin concentrations generally correlate well with tissue iron stores.

Influence of iron stores: Depletion of iron stores causes increased absorption and increased iron stores cause reduced absorption. In humans with iron deficiency, the amount of food iron absorbed increases from the usual 5–10 percent to 10–20 percent.

Influence of erythropoietic activity: An increase in erythropoiesis either due to hemolysis or hemorrhage increases absorption, while depression of erythropoiesis by transfusion-induced erythrocytosis decreases absorption.

The amount of iron lost from the body per day is between 0.5 and 1.0 mg under physiological conditions. This figure does not take into account loss by menstruation in the female. The rate of iron loss is relatively constant and is independent of intake, occurring as a result of the desquamation of epithelial cells (mainly from the alimentary tract, from excretion in the urine, sweat, and loss of hair and nails).

BAQ: How iron balance of the body is maintained?

Ans: Under normal circumstances, iron absorption slightly exceeds iron excretion. The uptake of iron from food is about 1–2 mg per day. Basal losses range from 0.5 to 1.0 mg per day. In females, due to menstruation, the monthly loss of iron is between 15 and 28 mg, i.e. between 0.5 and 1.0 mg per day (for a 28-day menstrual cycle). Thus, the daily absorption necessary to compensate for daily loss is 0.5–1.0 mg in males, and 1–2 mg in females (particularly during the reproductive period of life).

The daily iron requirement for hemoglobin synthesis is 20–25 mg. Most of this iron is provided by macrophages, recycling iron from destructed old red blood cells. In normal individuals, red cell destruction and formation take place at almost identical rates. Thus, in the absence of bleeding or increased demand, sufficient iron for hemoglobin synthesis is provided by the breakdown of hemoglobin during the destruction of aged red cells.

It has been estimated that 20 percent of the world's population is iron deficient and iron deficiency anemia is the most common type of anemia met with in clinical practice. It occurs at all ages but is especially common in women of childbearing age. IDA is always secondary to an underlying disorder. In industrialized communities, it is usually due to chronic and often occult blood loss. In the Third World, poor intake of iron or defective absorption are more frequent causes. Correction of the underlying cause is an essential part of the treatment.

Importance of Haemogram and Iron Panel Tests

Competency achievement: The student should be able to:

PE13.2: Describe the causes, diagnosis, and management of iron deficiency

Competency achievement: The student should be able to:

PE13.4: Describe the haemogram and iron panel

LAQ: Describe the clinical significance of hemogram and iron panel in the diagnosis of iron deficiency anemia (IDA).

Ans: The following are the tests related to complete haemogram and related clinical significance in IDA:

Hemoglobin: Significant decrease in blood hemoglobin (Male: <13 g, Female <12 g, Children < 11g)

Red blood cell (RBC) count: Significant decrease in RBC count (Males: < 4.5 × 10^6 / cmm, Females <4.0 × 10^6/cmm, Children < 3.5 × 10^6 /cmm)

Significant decrease in the following parameters as seen in stained blood smear and using PCV values:

Packed cell volume (PCV): < 36%

Mean corpuscular volume (MCV): <80 fl

Mean corpuscular hemoglobin (MCH): <27 µl

Mean corpuscular hemoglobin concentration (MCHC): <32 %

Red cell distribution width (RDW) >18

Increased white blood cell count (WBC) of more than 10,000 cell/cmm indicates bacterial infection. *Helicobacter pylori* infection may lead to the formation of ulcers in the stomach and related bleeding may cause IDA.

Increased eosinophils by more than 4% indicate the possibility of parasitic infection. Hookworm infection may lead to IDA.

Following are iron panel tests and related clinical significance in IDA:

In IDA, serum total iron, serum ferritin, and serum transferrin decrease significantly as follows:

1. Serum total iron: <60 µg/dl
2. Iron binding capacity: >380 µg/dl
3. Serum ferritin: <15 µg/dl
4. Serum transferrin: <204 µg/dl

A complete hemeogram gives an idea about iron deficiency on the following anatomical features and physiology:

Anatomy: Adverse effects on the complete anatomical structure, due to low oxygen-carrying capacity of red blood cells.

Body weight: Decrease in body weight.

Skin: Pale skin due to iron deficiency.

Body mass: Loss of body weight.

Neurons: Adverse effects on the functioning of the neurological system.

Bone marrow: Production of microcytes and adipocytes, due to iron deficiency.

Physiology: Reduced capacity of red blood cells to carry oxygen to the various cells of the body.

Reduced capacity of the patient to perform normal functions.

IMPORTANCE OF TRACE ELEMENTS

BAQ: What are trace elements? Give classification of trace elements.

Ans: The trace elements are minerals present in body fluids and living tissues in very small amounts.

The trace elements are classified as follows:

1. *Essential trace elements:* Iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium, chromium, and fluorine.
2. *Possibly essential trace elements:* Nickel, tin, vanadium, and silicon.
3. *Nonessential trace elements:* Aluminum, boron, germanium, cadmium, arsenic, lead, and mercury.

SAQ: Name any two essential trace elements and their importance in metabolic reactions.

Ans: Iron and zinc

1. Iron forms an important integral component of hemoglobin, myoglobin, and cytochromes.
2. Zinc is an essential component of several enzymes present in tissues, including alkaline phosphatase, carbonic anhydrase, alcohol dehydrogenase, etc. It is also an important component of insulin.

BAQ: Write the importance of the following trace elements: Manganese, iodine, copper, magnesium and sulfur.

Ans:

- Manganese is necessary for normal bone structure, reproduction, and normal functioning of the central nervous system.
- Iodine is required for the biosynthesis of the iodinated thyroid hormone.
- Copper is an essential constituent of several proteins, metalloenzymes, and some naturally occurring pigments.
- Magnesium is present in combination with calcium and phosphorus in the complex salt of bone. The remainder is present in soft tissues and body fluids.
- Sulfur is present in all the cells of the body, primarily in the cell protein in the form of two sulfur-containing amino acids, cysteine, and methionine.

SAQ: Write the importance of cobalt and fluoride as trace elements.

Ans: Cobalt is a constituent of vitamin B_{12}, necessary for the normal formation of red blood cells.

Fluorine plays an important role in the development of teeth and bones. Despite the toxic properties of fluoride at high concentrations, in trace quantities (1 ppm in drinking water), i.e. 1 to 2 mg of fluoride is ingested every day, according to the water intake in all forms.

Competency achievement: The student should be able to:

PE13.13: Discuss the RDA, dietary sources of magnesium and its role in health and disease

LAQ: Write a note on the importance of magnesium, magnesium deficiency symptoms, RDA, and treatment.

Ans: Magnesium is the second most prevalent intracellular cation and fourth most abundant cation in the body. The concentration of magnesium in cells is about 2.4–7.3 mg/dl. The high cellular activity requires high cellular content of magnesium. Within the cell, most of the magnesium is bound to proteins and negatively charged molecules. Cytosolic 80% magnesium is bound to ATP and Mg-ATP plays a very important role in enzyme-catalyzed reactions in the body. Magnesium is a cofactor for about 300 enzymes in the body. The average serum concentration of magnesium in adults is 1.6–2.6 mg/dl. Extracellular fluids contain about 1% of the total magnesium in the body. 30% magnesium is bound to albumin, 15% is bound to citrates, phosphates, and other ions, and 55% magnesium is free.

Magnesium is an essential mineral and electrolyte that plays a role in many metabolic reactions including energy production, contributing to the functions of muscles, role in maintaining normal bone and teeth structure, nerve functions, RNA and protein synthesis, DNA replication, etc. Hypermagnesemia (high serum magnesium) and magnesium intoxication are not frequently encountered clinical problems.

Magnesium deficiency may be created due to the following reasons: Low-magnesium diet, gastrointestinal disorders such as Crohn's disease, celiac disease, or regional enteritis, losing excessive amounts of magnesium through urine and sweat resulting from genetic disorders, drinking excessive alcohol, pregnancy, parathyroid disorders, hyperaldosteronism, type 2 diabetes mellitus, old age, intake of proton pump inhibitors, diuretics, bisphosphonates, and antibiotics.

Long-term magnesium deficiency may have adverse effects on:

Bone density, brain function, nerve and muscle function, and digestive system.

Recommended daily allowance (RDA): The Recommended dietary allowance (RDA) for adult males is 350 mg of magnesium per day and for adult females 300 mg per day.

For pregnant and lactating women, the RDA is 450 mg per day.

The ideal intake for magnesium should be based on body weight (6 mg per kg of body weight).

Food supplements: It is possible to reach the RDA for magnesium by eating foods that contain high levels of magnesium, such as green vegetables, fruit, whole grains, cereals, and legumes.

Some foods high in magnesium content, include: Nuts, especially almonds, cashews, peanuts, spinach, black beans, peanut butter, whole wheat bread,

Avocado, potato, rice, yogurt, fortified cereals, oatmeal, fish, such as salmon and halibut, milk, chicken breast, broccoli, and carrot also contain moderate amounts of magnesium.

BAQ: Write a note on magnesium absorption, factors, and conditions that affect magnesium absorption.

Ans: Magnesium absorption: Magnesium is absorbed principally in the small intestine, through a saturable transport system, and by passive diffusion through the flow of water. The absorption of magnesium depends on the amount ingested. When the dietary content of magnesium is normal, approximately 30–40% of magnesium is absorbed. Under conditions of low magnesium intake, approximately 80% is absorbed, while only about 25% is absorbed when the intake is high.

The following certain nutrients and conditions affect the absorption of magnesium:

1. Calcium-rich foods two hours before or after eating magnesium-rich foods
2. High-dose zinc supplements
3. Vitamin D deficiency
4. Raw vegetable intake

Magnesium and vitamin D depend on each other for function and absorption.

BAQ: Enumerate magnesium deficiency symptoms. Give information on laboratory tests to determine serum magnesium.

Ans: The following are magnesium deficiency symptoms: Fatigue, aches, pains muscle cramps, migraines, irregular sleep patterns, insomnia, heart irregularities, mood swings, malabsorption syndromes, acute and chronic diarrhea, etc.

Laboratory tests: Fluorometry, flame emission spectroscopy, and atomic absorption spectroscopy methods are used to measure serum magnesium concentration.

SAQ: Write a short note on magnesium supplements.

Ans: A physician may recommend magnesium supplements for patients who have poor magnesium absorption or an underlying health condition that may prevent sufficient magnesium intake.

Magnesium supplements are available in a variety of formulations, such as magnesium citrate, magnesium chloride, and magnesium oxide.

A person's body absorbs the magnesium from the citrate and chloride formulations more efficiently than the oxide form.

Competency achievement: The student should be able to:

DR17.4: Enumerate and describe the various changes in zinc deficiency

LAQ: Write a note on the importance of zinc, normal levels of serum zinc, zinc deficiency symptoms, RDA, and treatment.

Ans: Zinc is present within cells. The role of zinc can be grouped into three general functional classes: Catalytic, structural, and regulatory functions.

Zinc deficiency is more prevalent in areas of high cereal and low animal food consumption. The diet may not necessarily be low in zinc, however, its bio-availability plays a very important role in its absorption. Compared to adults, infants, children, adolescents, pregnant, and lactating women have increased requirements for zinc, and hence, these are at increased risk of zinc depletion.

Zinc deficiency during growth periods affects skeletal, epidermal, gastrointestinal, central nervous, reproductive, and immune systems.

Recommended daily allowance (RDA) of zinc:

For children (from 1 to 12 years of age): 5–9 mg

For males and females: 10–12 mg

Pregnant and lactating mothers: 12 mg

Laboratory tests for determination of zinc: The blood plasma or serum zinc concentration, dietary intake, and stunting prevalence are the best-known indicators of zinc deficiency. The main intervention strategies for combating zinc deficiency include dietary modification, supplementation, and fortification.

The normal range of serum/plasma zinc:

Between the ages of 1 and 12 months: 60–90 μg/dl

Between the ages of 1 and 10 years: 80–110 μg/dl

Between the ages of 10 and 15 years: 90–120 μg/dl

Adults: 80–120 μg/dl

Causes of zinc deficiency: The general causes of zinc deficiency include inadequate intake, increased requirements, malabsorption, increased losses, and impaired utilization. Inadequate dietary intake of absorbable zinc is the primary cause of zinc deficiency in most situations. This may result from low dietary intake or heavy intake of foods containing phytate, calcium, and iron.

Malabsorption syndromes and inflammatory diseases of the bowel also lead to poor absorption and loss of zinc. Utilization of zinc is impaired in the presence of infection as decreased circulation of zinc reduces the availability of zinc to the tissues.

Fecal excretion of zinc is increased during acute diarrhea. The zinc deficiency increases the susceptibility to childhood diarrhea, while increased losses of endogenous zinc associated with diarrhea further deplete body zinc.

Supplementation regime: Supplementation programs are useful for targeting vulnerable population subgroups, which are at a particularly high risk of micronutrient deficiencies. The recommended zinc dosages are 5 mg/day for children between 7 months and 3 years and 10 mg/day for older children. When formulating multi-nutrient supplements, it is recommended that salts providing readily absorbable zinc, like $ZnSO_4$, zinc gluconate, or zinc acetate are used because they are absorbed more efficiently.

Supplemental zinc is also recommended as an adjunct therapy during the treatment of diarrhea in children. The recommended daily dosage is twice the age-specific RDA per day for 14 days. That means, 10 mg/day for children under 3 years and 20 mg for older children.

LAQ: Write a note on the absorption, transport, metabolism, and excretion of zinc.

Ans: Zinc is released from food as free ions during digestion. These liberated ions then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum. Specific transport proteins may facilitate the passage of zinc across the cell membrane into the portal circulation. With high intake, zinc is also absorbed through a passive paracellular route.

Zinc is absorbed in the small intestine by a carrier-mediated mechanism. Under normal physiologic conditions, transport processes of uptake are not saturated. Zinc is also secreted into the gut. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60–70%), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition. Generally, 33% zinc is accepted as the average zinc absorption in humans. Zinc absorption is concentration-dependent and increases with increasing dietary zinc up to a maximum rate. Zinc-deprived persons absorb zinc with increased efficiency, whereas persons on a high-zinc diet show a reduced efficiency of absorption.

The portal system carries absorbed zinc directly to the liver. It is then released into systemic circulation for delivery to other tissues. About 70% of the zinc in circulation is bound to albumin, and any condition that alters serum albumin concentration can have a secondary effect on serum zinc levels. Although serum zinc represents only 0.1% of the whole body's zinc, the circulating zinc turns over rapidly to meet tissue needs.

In the liver, zinc is incorporated into metalloenzymes, albumin, and alpha-2-macroglobulin. 80% zinc is associated with serum albumin and it is in equilibrium with serum amino acids. 20% zinc is tightly bound to alpha-2-macroglobulin. The total adult body content of zinc is about 2–2.5 g and it is present in metabolically active tissues and organs. About 55% of zinc is found in muscle and 30% in bones. Loss of zinc through the gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. A considerable amount of zinc is secreted through the biliary and intestinal secretions, however, most of it is reabsorbed. This is an important process in the regulation of zinc balance. Other routes of zinc excretion include urine and surface losses (desquamated hair, skin, and sweat).

BAQ: What is the bioavailability of zinc? What factors affect the absorption of zinc?

Ans: Bioavailability of zinc refers to the fraction of intake that can be absorbed into the blood system and used for physiologic functions of the body. In healthy individuals, it is determined by three factors: The individual zinc status, the total zinc content of the diet, and the availability of soluble zinc from the diet's food components.

Zinc absorption is largely determined by its solubility in the intestinal lumen, which in turn is affected by the chemical form of zinc and the presence of specific inhibitors and promoters of zinc absorption.

Long-term zinc intake can affect the absorption of dietary zinc. About 30% of zinc in the diet is absorbed in the small intestine. Constituents in food such as calcium, phytate, and iron reduce the absorption of zinc.

SAQ: What is the RDA of zinc?

Ans: Recommended daily allowance (RDA) of zinc is as follows:

For children (from 1 to 12 years of age): 5–9 mg

For males and females: 10–12 mg

Pregnant and lactating mothers: 12 mg

SAQ: What is the normal range of serum zinc?

Ans: The normal range of serum zinc is as follows:

Between the ages of 1 and 12 months: 60–90 µg/dl

Between the ages of 1 and 10 years: 80–110 µg/dl

Between the ages of 10 and 15 years: 90–120 µg/dl

Adults: 80–120 µg/dl

BAQ: Enumerate the causes of zinc deficiency.

Ans: The general causes of zinc deficiency include inadequate intake, increased requirements, malabsorption, increased losses, and impaired utilization.

Inadequate dietary intake of absorbable zinc is the primary cause of zinc deficiency in most situations. This may result from low dietary intake or heavy intake of foods containing phytate, calcium, and iron.

Malabsorption syndromes and inflammatory diseases of the bowel also lead to poor absorption and loss of zinc. Utilization of zinc is impaired in the presence of infection as decreased circulation of zinc reduces the availability of zinc to the tissues. Malabsorption of zinc occurs in acrodermatitis enteropathica. In this clinical condition, zinc absorption is genetically affected.

Conditions of impaired intestinal integrity not only reduce absorption but also result in increased endogenous losses of zinc. Fecal excretion of zinc is increased during acute diarrhea. The zinc deficiency increases the susceptibility to childhood diarrhea, while increased losses of endogenous zinc associated with diarrhea further deplete body zinc.

BAQ: Write a note on supplementation and food fortification regime for zinc.

Ans: Supplementation programs are useful for targeting vulnerable population subgroups, which are at a particularly high risk of micronutrient deficiencies. The recommended zinc dosages are 5 mg/day for children between 7 months and 3 years and 10 mg/day for older children. When formulating multinutrient supplements, it is recommended that salts providing readily absorbable zinc, like $ZnSO_4$, zinc gluconate, or zinc acetate are used because they are absorbed more efficiently.

Supplemental zinc is also recommended as an adjunct therapy during the treatment of diarrhea in children. The recommended daily dosage is twice the age-specific RDA per day for 14 days. That means, 10 mg/day for children under 3 years and 20 mg for older children. Several clinical trials have demonstrated that zinc supplements reduce the severity and duration of acute and persistent diarrhea.

Food fortification: Food fortification is a more cost-effective and sustainable strategy to overcome micronutrient malnutrition. Where micronutrient deficiency is widely distributed in a population; fortification of centrally processed foods is an appropriate alternative. Apart from zinc, other micronutrients are added to wheat and corn flour that are used in the preparation of bread.

Fortification programs are also specifically targeted to increase the intake of zinc in groups of high-risk such as infants and young children who consume particular types of food. Commercially available standard infant formulas contain zinc in concentrations of around 1 mg/L, following current recommendations. Among several zinc compounds that are available for fortification, zinc oxide, and $ZnSO_4$ are the least expensive and most commonly used by the food industry. Suggested levels for fortification of flour are 30–70 mg zinc/kg.

Competency achievement: The student should be able to:

PE13.7: Discuss the RDA, dietary sources of iodine and their role in health and disease

Competency achievement: The student should be able to:

PE13.8: Describe the causes, clinical features, diagnosis, and management of deficiency of iodine

Competency achievement: The student should be able to:

PE13.9: Identify the clinical features of iodine deficiency

LAQ: Write a note on the importance of iodine, RDA, dietary sources, digestion, absorption, deficiency symptoms, and treatment regime.

Ans: As an essential mineral, iodine is used by the thyroid gland to make thyroid hormones that control many functions in the body including growth and development. Iodine needs to be supplied in the diet. When iodine intake is poor, the body cannot produce enough thyroid hormones.

Iodine deficiency in pregnancy is a worldwide problem and has become a global public health concern. It is identified as the leading cause of preventable brain damage in newborns and infants. Iodine deficiency is a common problem in underdeveloped countries. Hypothyroidism, thyroid gland enlargement (goiter), and weight gain are other conditions that may result from a deficiency of iodine in the diet. Brain damage, cretinism, mental retardation, and other conditions are additional risk factors for iodine deficiency. However, iodine can be significantly improved with the intake of iodine as per recommended daily allowance (RDA).

Iodine Recommended Daily Allowance (RDA):

Following are the recommended dietary allowances for iodine:

1 to 8 years old: 90 µg
9 to 13 years old: 120 µg
14 years and older: 150 µg
Pregnant female: 220 µg
Lactating female: 290 µg

Best food sources of iodine: Saltwater fish, seaweed, and seafood are natural sources of dietary iodine. Dairy products also supply iodine in the diet at varying levels. During lactation, the breast concentrates iodine in milk. Hence, breast milk tends to be a good source of iodine as long as the mother's iodine intake is adequate.

Digestion and absorption of iodine: Iodine can be bound to amino acids, or it can be free, usually in the form of iodate or iodide ions. Iodide is the easiest form to absorb, so most of the bound iodine and iodate is converted to iodide by glutathione. The iodide ions are easily absorbed through the walls of the digestive tract in the stomach and small intestine. After it is absorbed, most of it concentrates in the thyroid gland. Some of it also accumulates in the ovaries, skin, and salivary, gastric, and mammary glands.

Iodine deficiency symptoms: Enlarged thyroid gland (Goiter), weakness, fatigue, dry skin, slow pulse rate, learning and memory difficulties.

Iodine deficiency treatment: Iodine deficiency is treated by providing foods that contain iodine, using iodized salt, and using iodine supplements. Multivitamins often contain iodine in the form of potassium iodide or sodium iodide. Supplements containing kelp (seaweed) are a good source of iodine. However, supplements should be taken with the advice of a physician.

SAQ: What is iodine fortification?

Ans: Iodine fortification is what most countries rely on to encourage adequate dietary intake. In more than 70 countries iodized salt generally serves as the major source of iodine intake. One-fourth of a teaspoon of iodized salts has about 100 µg of iodine. Iodized salt usually adds about 300 µg of iodine daily to the diet. Most multivitamin-mineral supplements contain 150 µg of iodine.

BAQ: Give available iodine obtained from iodized salt, egg, seaweed, reduced-fat milk, white-enriched milk, and cheddar cheese.

Ans: Available iodine (DV: Daily Value) from the following listed food items:

- *Iodized salt:* A quarter teaspoon, or 1.5 g, contains 71 µg, or 47 percent of daily value (DV).
- *Egg:* One large egg contains 24 µg or 16 percent of DV.
- *Seaweed:* 1 gram (g) of whole or sheet seaweed contains from 16 to 2,984 µg of iodine.
- *Reduced-fat milk:* 1 cup contains 56 µg or 37 percent of DV.
- *White, enriched bread:* 2 slices contain 45 µg or 30 percent of DV.
- *Cheddar cheese:* 1 ounce contains 12 µg or 8 percent of DV.

SAQ: What are goitrogens? Give examples of goitrogens.

Ans: Goitrogens are naturally-occurring chemicals found in many plant-based foods.

Consuming high amounts of these substances regularly may harm thyroid health. Examples are turnips, cassava, soy, broccoli, cabbage, etc. However, cooking can inactivate these goitrogens.

LAQ: Write a note on iodine metabolism and synthesis of thyroid hormones.

Ans: The normal daily intake of iodine is 100–200 µg. It is absorbed mainly in the small intestine and transported in the plasma in loose attachment to the proteins.

About two-thirds of the ingested iodine is excreted by the kidneys, and the remaining one-third is taken up by the thyroid gland.

TSH (thyroid stimulating hormone) of the pituitary stimulates iodine uptake by the gland. Within the thyroid, iodine is oxidized and transferred to tyrosine molecules in thyroglobulin (a glycoprotein) by peroxidase. This reaction is also stimulated by TSH. Iodination of tyrosine leads to the formation of monoiodotyrosine (I_1Tyr) and diiodotyrosine (I_2Tyr)

The coupling of 2 molecules of I_2Tyr then occurs within the thyroglobulin molecule to form tetraiodothyronine or thyroxine (T_4), and the coupling of I_1Tyr and I_2Tyr leads to the formation of triiodothyronine (T_3). In addition, small amounts of biologically inactive reverse triiodothyronine also form.

About 80% of the iodine stored by the thyroid gland is thyroxine, and 20% is probably T_3. Conversion of inorganic iodine to T_4 and T_3 takes about 48 hours.

The release of T_4 and T_3 takes place by (A) A secretion process involving microtubules and microfilaments. (B) Hydrolysis of thyroglobulin by a protease is stimulated by TSH.

Within the plasma, T_4 and T_3 are transported almost entirely in association with thyroxine-binding globulin (TBG) and thyroxine-binding pre–albumin. When the binding capacities of these proteins have exceeded the hormones, they bind to serum albumin.

T_3 is 3–5 times more active than T_4 and has a more rapid onset of action. Both T_4 and T_3 are metabolized in the peripheral tissues by deamination and decarboxylation. They perform the following functions:

A. As a catalyst for the oxidative reactions and regulation of metabolic rates in the body.

B. Enhance oxygen uptake and lipolysis and decrease circulating cholesterol.

C. Accelerate anabolic reactions by causing an increase in RNA and protein synthesis, which precedes basal metabolic rate (BMR).

D. Increase ATP utilization.

Deiodination of T_4 and T_3 may occur in peripheral tissues. About 40% of secreted T_4 is deiodinase to T_3, and about 45% is deiodinated to rT_3 (reverse T_3). The liberated iodine is then excreted in the urine.

In the liver, T_3, and T_4 rapidly conjugate with glucuronic acid, and the conjugates are then excreted into the bile. Part of the conjugate may be reabsorbed in the small intestine, and then it is excreted in the urine.

Although the major portion of iodine is present in the thyroid gland, the non-hormonal iodine is found in a variety of body tissues including salivary glands, gastric mucosa, mammary glands, eye, and cervix. Iodine in the breast plays an important role during breastfeeding in fetal and neonatal development.

BAQ: What is the anion gap and its clinical significance?

Ans: The anion gap is the difference between the total number of cations and the total number of anions in a specimen. It is calculated mainly by subtracting concentrations of chloride and bicarbonate (anions) from the concentrations of sodium and potassium(cations) as follows: $\{(Na^+) + (K^+)\} - \{(Cl^-) + (HCO_3^-)\}$.

Concentrations of Ca^{++}, Mg^{++}, H_2PO, etc. in serum are very low, hence these are not considered usually. The normal anion gap is

3–11 mEq/L when a freshly collected serum is used (within 30 minutes of blood collection).

A high anion gap indicates a significant loss of either chloride ions or bicarbonate ions. A high anion gap indicates the development of acidosis. In diabetic keto-acidosis decrease in bicarbonate fraction (due to ketone bodies) anion gap increases significantly. In renal failure, due to the loss of bicarbonate, the anion gap increases. In prolonged vomiting, although there is a significant loss of chloride ions, since bicarbonate increases in serum, the anion gap remains normal.

A low anion gap is observed in hypoalbuminemia. Loss of negatively charged albumin in urine leads to retention of other negatively charged ions such as bicarbonate and chlorides. In multiple myeloma, the anion gap is reduced due to an increase in serum IgG.

Plasma Cl^- is affected by changes in Na^+ and HCO_3^-. An increased value of chloride is seen in metabolic acidosis in which there is a loss of HCO_3^-. Similarly, in metabolic alkalosis (prolonged vomiting), when HCO_3^- values are increased in the blood, very low values of chloride are observed.

BAQ: Explain Gibbs-Donnan law with one example.

Ans: According to the Gibbs-Donnan law, if solutions on two sides of a membrane contain different concentrations of ions that cannot move through the membrane (e.g. proteins), the distribution of electrolytes (diffusible ions) at the steady state will be unequal. However, the product of ions in one compartment is equal to the product of ions in another compartment. Similarly, the law of electrical neutrality is observed by both compartments.

The Gibbs-Donnan law is also important in establishing the distribution of ions and solutes across the capillary endothelium in tissue capillaries. The capillary endothelium is not permeable to large proteins but permeable to ions and small solutes.

An example of uneven distribution of an ion with different protein content is the concentration of chloride ions in plasma and CSF. Due to the selectivity of the blood–brain barrier against proteins, Cl^- ions are about 15% higher in CSF compared to plasma, to establish osmotic and electrical equilibrium.

Multiple Choice Questions

Q1. Which clinical condition may lead to severe loss of body fluids?

A. Gastroenteritis
B. Fanconi syndrome
C. Nephrogenic diabetes insipidus
D. Renal failure

Q2. The daily normal water intake for the normal average adult is about

A. 1800–2500 ml
B. 400–800 ml
C. 700–1000 ml
D. 300–600 ml

Q3. The predominant cation of serum (plasma) is

A. K^+
B. Ca^+
C. Na^+
D. Mg^{++}

Q4. The predominant anion of serum (or plasma) is

A. HPO_4^-
B. HCO_3^-
C. Cl^-
D. SO_4^-

Q5. The daily total body water derived from metabolic reactions is about

A. 50–80 ml
B. 80–140 ml
C. 200–300 ml
D. 400–650 ml

Q6. Primary dehydration may lead to

A. Increase in intracellular fluid volume
B. Decrease in intracellular fluid volume
C. Increase in extracellular fluid
D. None of the above

Q7. An important cause of secondary dehydration

A. Gastroenteritis
B. Prehepatic condition
C. Fructosuria
D. Postrenal condition

Q8. Secondary dehydration may lead to

A. Intracellular edema
B. Cellular dehydration
C. Tetany
D. B and C

Q9. The total calcium in the human body is about

A. 1.0–1.5 kg B. 3.0–4.5 kg
C. 500–750 g D. 300–500 g

Q10. Daily requirement of calcium for a normal adult is about

A. 300–400 mg B. 0.5–1.0 g
C. 50–100 mg D. 3.0–4.0 g

Q11. Normal range (reference range) of total serum calcium is

A. 4.8–6.5 mg/dl
B. 8.5–10.5 mg/dl
C. 10–15 mg/dl
D. 2.0–2 .5 mg/dl

Q12. Which mineral is present in the human body in larger amounts than any other cation?

A. Magnesium B. Sodium
C. Potassium D. Calcium

Q13. The percentage of calcium present in the extracellular fluid is about

A. 40 B. 10 C. 25 D. 60

Q14. Which of the following is the physiologically active form of calcium?

A. Ionized
B. Protein bond
C. Combined with carbonate
D. Combined with citrate

Q15. The normal range of CSF calcium is

A. 4.5–5.0 mg/dl B. 2.5–4.0 mg/dl
C. 7.5–14.0 mg/dl D. 10–15 mg/dl

Q16. Absorption of calcium is increased on a

A. High-fat diet
B. High protein diet
C. Low carbohydrate diet
D. Low-fat diet

Q17. Calcium absorption is interfered by

A. Carbohydrates in diet
B. Phytic acid in cereals
C. Protein in diet
D. Vitamin D

Q18. Vitamin that increases calcium absorption in the intestine is

A. Vitamin A B. Vitamin C
C. Vitamin D D. Biotin

Q19. Renal rickets is caused by renal tubular defect due to interference with the reabsorption of

A. Calcium B. Phosphorus
C. Potassium D. Chloride

Q20. The normal range of serum (or plasma) sodium is

A. 100–120 mEq/L
B. 200–250 mEq/L
C. 50–60 mEq/L
D. 133–146 mEq/L

Q21. A decrease in serum sodium may occur in

A. Cushing's syndrome
B. Hyperparathyroidism
C. Hyperthyroidism
D. Addison's disease

Q22. Hypernatremia may occur in

A. Addison's disease
B. Cushing's syndrome
C. Hepatic jaundice
D. Kidney disease

Q23. The metabolism of sodium is regulated by

A. PTH B. Glucagon
C. Aldosterone D. Insulin

Q24. The principal cation in intracellular fluid is

A. Calcium B. Sodium
C. Potassium D. Magnesium

Q25. The normal range of serum (or plasma) potassium in human plasma is

A. 2.0–3.0 mEq/L B. 3.0–4.0 mEq/L
C. 1.5–2.5 mEq/L D. 3.8–5.6 mEq/L

Q26. Following is one of the symptoms of low serum potassium concentration

A. Anorexia B. Numbness
C. Confusion D. Muscle weakness

Q27. Hypokalemia occurs in

A. Rickets
B. Addison's disease
C. Diabetes insipidus
D. Cushing's syndrome

Q28. Overdoses of which of the following cations may lead to cardiac arrest

A. Potassium B. Calcium
C. Sodium D. Magnesium

Q29. The normal range of plasma chloride is

A. 80–120 mEq/L B. 50–65 mEq/L
C. 95–106 mEq/L D. 130–145 mEq/L

Q30. The normal range of CSF chloride is

A. 50–65 mg/dl
B. 100–150 mg/dl
C. 200–250 mg/dl
D. 700–750 mg/dl

Q31. Hyperchloremia is associated with

A. Metabolic alkalosis
B. Hyponatremia
C. Hypernatremia
D. Respiratory acidosis

Q32. The normal range of plasma bicarbonate is

A. 5–7 mEq/L
B. 10–15 mEq/L
C. 21–26 mEq/L
D. 30–37 mEq/L

Q33. Chloride shift means

A. Chloride ions leave the RBC in exchange for bicarbonate
B. Hydrogen ions leave the RBC in exchange for chloride ions
C. Bicarbonate ions enter red blood cells in exchange for sodium ions
D. Bicarbonate ion returns to plasma and is exchanged with chloride which shifts into the red blood cell

Q34. Which of these are osmotically active substances in plasma?

A. Potassium
B. Sodium
C. Proteins
D. A, B, and C

Q35. Contribution of albumin to colloid osmotic pressure of plasma is about

A. 80%
B. 60%
C. 20%
D. 50%

Q36. Oncotic pressure of plasma is mainly due to

A. Bicarbonate ions
B. Proteins
C. Sodium ions
D. All of these

Q37. Oncotic pressure of plasma is about

A. 50 mm of Hg
B. 28 mm of Hg
C. 5 mm of Hg
D. 35 mm of Hg

Q38. This may lead to edema, when,

A. Plasma Na^+ and Cl^- are increased significantly
B. Plasma Na^+ and Cl^- are decreased significantly
C. Plasma proteins are increased significantly
D. Plasma proteins are decreased significantly

Q39. Prolonged vomiting leads to loss of

A. Chloride
B. Bicarbonate
C. Sodium
D. All of these

Q40. Prolonged vomiting leads to an increase in these ions in the blood

A. Chloride
B. Bicarbonate
C. Sodium
D. All of these

Q41. Urinary water loss is increased in

A. Diabetes mellitus
B. Diabetes insipidus
C. Prerenal conditions
D. A and B

Q42. Diabetes insipidus occurs due to

A. Decreased insulin secretion
B. Increased ADH secretion
C. Decreased ADH secretion
D. A and B

Q43. Thiazide diuretics inhibit

A. Sodium reabsorption in distal tubules
B. Calcium absorption in distal tubules
C. Calcium excretion in distal tubules
D. Sodium excretion in distal tubules

Q44. Furosemide inhibits the reabsorption of sodium and chloride in

A. Collecting ducts
B. Distal convoluted tubules
C. Loop of Henle
D. Proximal convoluted tubules

Q45. Anion gap is mainly the difference in the serum (plasma) concentrations of

A. (Sodium + Potassium) – (Chloride + Bicarbonate)
B. (Sodium) – (Chloride)
C. (Chloride) – (Bicarbonate)
D. (Sum of cations) – (Sum of anions)

Q46. Normal anion gap in serum (plasma) is about

A. 15 mEq/L
B. 5 mEq/L
C. 20 mEq/L
D. 45 mEq/L

Q47. Anion gap is normal in

A. Diabetic ketoacidosis
B. Hyperchloremic metabolic acidosis
C. Lactic acidosis
D. Uremic acidosis

Q48. Anion gap may increase in

A. Diabetic ketoacidosis
B. Metabolic acidosis resulting from diarrhea
C. Metabolic acidosis resulting from intestinal obstruction
D. Renal tubular acidosis

Q49. A man was trapped in a collapsed building. He did not have any injury and there was no blood loss. He had no access to water or food until he was rescued after 64 hours. He required emergency treatment due to

A. Decrease in ICF
B. Decrease in ECF
C. Possibility of circulatory failure
D. All of the above

Q50. A 52-year-old woman was admitted to a hospital with a two-day history of severe diarrhea. She was weak and was not able to stand. Her intake in the past 2 days was only water. Which of the following is the most appropriate emergency treatment for the patient?

A. Intravenous (IV) glucose saline
B. Oral electrolyte solution
C. Only appropriate drug therapy
D. Appropriate drug therapy following IV–Glucose saline

Answers

1. A	**2.** A	**3.** C	**4.** C	**5.** C	**6.** B	**7.** A	**8.** B	**9.** A	**10.** B
11. B	**12.** D	**13.** B	**14.** A	**15.** A	**16.** B	**17.** B	**18.** C	**19.** B	**20.** D
21. D	**22.** B	**23.** C	**24.** C	**25.** D	**26.** D	**27.** D	**28.** A	**29.** C	**30.** D
31. C	**32.** C	**33.** D	**34.** D	**35.** A	**36.** B	**37.** B	**38.** D	**39.** A	**40.** B
41. D	**42.** C	**43.** A	**44.** C	**45.** A	**46.** A	**47.** B	**48.** B	**49.** D	**50.** D

47. B. In hyperchloremic metabolic acidosis, a reduction in serum bicarbonate is compensated by an approximately equivalent increase in the serum chloride concentration resulting in low serum bicarbonate and high serum chlorides in the absence of an increase in the serum anion gap.

49. D. The man trapped in the collapsed building did not have access to fluids and water for 64 hours. Hence, there could be (A) A decrease in ICF (B) A decrease in ECF and (C) Possibility of circulatory failure.

50. D. Severe diarrhea for 2 days lead to significant body fluid loss. To compensate for this body fluid loss it was necessary to treat the patient with (D) Appropriate drug therapy following IV–Glucose saline

Case Studies

Case 1: A 22-year-old female student was admitted to the hospital due to severe diarrhea and vomiting, leading to declined general condition. Her blood electrolyte values were as follows:

	Reference range (Normal range)
Serum sodium: 123 mEq/L	133–146 mEq/L
Serum potassium: 3.1 mEq/L	3.8–5.6 mEq/L
Serum chlorides: 88 mEq/L	95–106 mEq/L

1. What is the probable diagnosis?

Ans: Hyponatremia and hypokalemia. Low levels of serum sodium, potassium, and chlorides.

2. What is the biochemical basis for a decrease in serum sodium, potassium and chlorides?

Ans: Loss of sodium, potassium, and chlorides was due to excessive loss of body fluids due to diarrhea and vomiting.

3. Why the patient suffered from diarrhea and vomiting?

Ans: Probably due to microbial infection (may be food poisoning).

4. What is the probable first line of treatment?

Ans: It is necessary initially to control diarrhea using drugs like loperamide, the action of which decreases the flow of fluids and electrolytes into the bowel and slows down the movement of the bowel. Similarly, adequate electrolytes are given to the patient to restore levels of serum sodium, potassium, and chlorides. If bacterial or parasitic infection is suspected, then an appropriate drug is prescribed according to the stool examination laboratory test report.

5. What are the additional laboratory tests required to confirm the diagnosis?

Ans: Routine stool examination and if necessary, stool antibiotic sensitivity test, before starting antibiotic treatment.

BAQ: Show horizontal integration of symptoms and test reports of Case 1 with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Infection of the small intestine

Horizontal integration with physiology

Increase in the intestinal mobility

Horizontal integration with nutrition

Feeding of adequate electrolyte drinks and use of electrolyte drinks and if necessary, saline drips.

BAQ: Show vertical integration of symptoms and test reports of Case 1 with pharmacology microbiology and preventive medicine

Ans: Vertical integration with pharmacology

Study and use of appropriate drugs to control intestinal mobility and specific drugs on microbial infections.

Vertical integration with microbiology

Study of gastrointestinal microbial and parasitic infections using stool specimens.

Vertical integration with preventive medicine

Preventive medicine: Study of education of safe food habits.

Case 2: A 58-year-old woman visited a physician with severe muscular weakness. She had a habit of taking large amounts of purgatives. Her blood examination reports were as follows:

	Reference range **Normal range**
Serum sodium: 130 mEq/L	133–146 mEq/L
Serum potassium: 2.3 mEq/L	3.8–5.6 mEq/L
Serum chlorides: 98 mEq/L	95–106 mEq/L

1. What is the probable diagnosis?

Ans: Drug-induced hypokalemia. Low levels of serum potassium.

2. What is the biochemical basis for a decrease in serum potassium?

Ans: Frequent loss of body fluids (containing electrolytes) due to excessive use of purgatives

3. What is the biochemical basis for severe muscular weakness?

Ans: Moderate hypokalemia is often associated with weakness, malaise, cramping, and myalgias.

4. What is the probable first line of treatment?

Ans: Immediate stoppage of purgatives. Most cases of mild-to-moderate hypokalemia may be corrected with oral (or intravenous) potassium supplements. Potassium chloride (KCl) is the most common salt used for repletion. It is also necessary to increase dietary intake of potassium-rich foods, such as bananas, potatoes, dried fruits, nuts, spinach, etc.

BAQ: Show horizontal integration of symptoms and test reports of Case 2 with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Severe muscular weakness

Horizontal integration with physiology

Electrolyte imbalance

Horizontal integration with nutrition

Feeding adequate electrolyte food and drinks rich in potassium and drips, if necessary.

BAQ: Show vertical integration of symptoms and test reports of Case 2 with pharmacology, preventive medicine and pathology.

Ans: Vertical integration with pharmacology
Study of drugs and supplements that support adequate intake of vitamin D.

Vertical integration with preventive medicine
Preventive medicine: Education of appropriate vitamin-rich foods to prevent diseases such as rickets.

Vertical integration with pathology
Study of cause of rickets, pathophysiology and prognosis.

Case 3: A 4-year-old boy was reported to a physician with complaints of severe weakness in the limbs and legs. He was unable to walk without support. His blood examination reports were as follows:

	Reference range (Normal range)
Serum calcium: 7.6 mg/dl	9–11 mg/dl
Serum inorganic phosphorus: 2.5 mg/dl	4.0–7.0 mg/dl
Serum alkaline phosphatase: 410 IU	93–221 IU

1. What is the probable diagnosis?

Ans: Rickets. Low levels of serum calcium, inorganic phosphorus, and very high levels of serum alkaline phosphatase indicate that he is suffering from rickets.

2. What is the biochemical basis for a decrease in serum calcium and inorganic phosphorus?

Ans: Vitamin D deficiency.

3. What is the biochemical basis for an increase in serum alkaline phosphatase?

Ans: Due to the increased osteoblastic activities in rickets. Osteoblast—the bone cells that become overactive when sufficient calcium and inorganic phosphorus are not available for new bone formation.

4. What is the probable line of treatment?

Ans: Intake of foods rich in vitamin D and vitamin D supplements. Similarly, exposure to early morning sun for at least 30 minutes.

BAQ: Show horizontal integration of symptoms and test reports of Case 3 with pharmacology and preventive medicine

Ans: Horizontal integration with anatomy
Severe weakness in the limbs and legs. Defects in the normal development of bones.

Horizontal integration with physiology
Low serum calcium and phosphorus due to vitamin D deficiency and disturbed metabolism.

Horizontal integration with nutrition
Feeding of foods containing vitamin D, such as milk, cheese, eggs, fish, meat, etc.

Show vertical integration of symptoms and test reports of Case 3 with pharmacology and preventive medicine.

Vertical integration with pharmacology
Study of drugs and supplements that support adequate intake of vitamin D.

Vertical integration with preventive medicine
Preventive medicine: Education of appropriate vitamin-rich foods to prevent diseases such as rickets.

Vertical integration with pathology
Study of cause of rickets, pathophysiology and prognosis

Case 4: A 36-year-old woman suffered from a couple of episodes of ureteric colic. Her blood examination reports were as follows:

	Reference range Normal range
Serum calcium: 12.6 mg/dl	9–11 mg/dl
Serum inorganic phosphorus: 1.5 mg/dl	2.5–5.0 mg/dl
Parathyroid hormone (PTH): 165 ng/l	10–65 ng/l

1. What is the probable diagnosis?

Ans: Ureteric colic is probably due to the formation of urinary calculi in the urinary tract. High levels of serum calcium, low levels of inorganic phosphorus, and very high levels of PTH indicate that she was suffering from **hyperparathyroidism**. In secondary hyperparathyroidism, PTH increases due to other reasons that lead to calcium loss like vitamin D deficiency and renal disease.

2. What is the biochemical basis for an increase in serum calcium and a decrease in inorganic phosphorus?

Ans: Hypersecretion of parathyroid glands

3. What is the mechanism for the increase in serum PTH?

Ans: Probably due to increased mass of parathyroid glands or due to adenoma (noncancerous growth), rarely due to cancerous growth.

4. What is the probable line of treatment?

Ans: Surgical removal of affected PTH glands

BAQ: Show horizontal integration of symptoms and test reports of Case 4 with anatomy, physiology and nutrition

Ans: Horizontal integration with anatomy

Hypersecretion of the affected parathyroid gland is probably due to adenoma.

Horizontal integration with physiology

Increased circulation of calcium and phosphorus due to hypersecretion of PTA.

BAQ: Show vertical integration of symptoms and test reports of Case 4 with general medicine and general surgery.

Ans: Vertical integration with general medicine

Study of parathyroid gland diseases.

Vertical integration with general surgery

Consideration of surgery to remove affected parathyroid glands

Case 5: A 53-year-old woman was admitted to the hospital due to a complaint of failing vision. She had undergone a thyroidectomy 15 years earlier. Her blood examination reports related to parathyroid gland functions were as follows:

	Reference range (Normal range)
Serum calcium: 6.4 mg/dl	9–11 mg/dl
Serum inorganic phosphorus: 8.5 mg/dl	2.5–5.0 mg/dl
Serum alkaline phosphatase (SALP): 68 IU	20–80 IU

1. What is the probable diagnosis?

Ans: Primary hypoparathyroidism. Low levels of serum calcium and high levels of inorganic phosphorus indicate that she was suffering from hypoparathyroidism. Diagnosis of rickets could be ruled out since inorganic phosphorus values are high and serum alkaline phosphatase values were normal.

There is no mention of the normal establishment of parathyroid glands after the thyroidectomy.

2. What is the biochemical basis for a decrease in serum calcium and an increase in inorganic phosphorus?

Ans: Deficiency of parathyroid hormones.

3. What is the mechanism for the decrease in serum PTH?

Ans: Due to thyroidectomy, which usually leads to removal and re-establishment of parathyroid glands. May be functions of the re-established parathyroid gland were not normal or delayed.

4. Why her vision was failing?

Ans: Due to the deposition of increased inorganic phosphorus on the retina of the eyes.

5. What is the probable line of treatment?

Ans: Oral calcium and vitamin D supplements, thiazide diuretics, and parathyroid hormone replacement therapy.

BAQ: Show horizontal integration of symptoms and test reports of Case 5 patient with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Affected parathyroid glands due to thyroidectomy.

Horizontal integration with physiology:

Low levels of serum calcium and phosphorus

BAQ: Show vertical integration of symptoms and test reports of Case 5 with general medicine and general surgery

Ans: Vertical integration with general medicine

Study of effects of thyroidectomy on parathyroid glands.

Vertical integration with general surgery

Consideration of successful re-establishment of parathyroid glands

Chapter

9

Acid–Base Balance

SPQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case studies: Marks: 4 or 5

INTRODUCTION

The pH of peripheral blood is normally within the range of 7.36 to 7.42. Arterial blood (pH 7.40) is more alkaline than venous blood (pH 7.37). On a mixed diet, the metabolic processes cause an overall production of acid. The body contains buffering mechanisms. After intermediate buffering in the cells, final compensation for any change in the hydrogen ion concentration of the extracellular fluid is performed by the lungs and kidneys. The oxidation of sulfur-containing amino acids and organic phosphates produces sulfuric acid and phosphoric acid, respectively. Sulfate, phosphate, and hydrogen ions are excreted by the kidneys.

The carbon dioxide formed in the metabolic reactions exists in blood in three main fractions: (1) As a small amount of carbonic acid (carbon dioxide dissolved in water), (2) The carbamino-bound fraction transported in combination with protein (mainly hemoglobin) and (3) That carried as bicarbonate in combination with the cations, sodium, and potassium. It is estimated that in 24 hours, the lungs remove the equivalent of 20–40 liters of 1 N acid as carbonic acid from the circulated venous blood, with the help of the enzyme carbonic anhydrase, which converts carbonic acid into carbon dioxide and water.

MAINTENANCE OF ACID–BASE BALANCE

Competency achievement: The student should be able to:

BI6.7: Describe the processes involved in the maintenance of normal blood pH, water, and electrolyte balance of body fluids and the derangements associated with these

LAQ: What is the normal range of blood pH? Describe general metabolic procedures that maintain normal blood pH.

Ans: The pH of peripheral blood, measured as the pH of plasma in contact with red cells, is normally within the very narrow range of 7.36 to 7.42. Arterial blood (pH 7.40) is more alkaline than venous blood (pH 7.37).

On a mixed diet, the metabolic processes cause an overall production of acid. The oxidation of sulfur-containing amino acids and organic phosphates produces sulfuric acid and phosphoric acid, respectively. Sulfate, phosphate, and hydrogen ions are excreted by the kidneys.

At the normal pH of blood (7.40), a ratio of 20:1 must exist between the bicarbonate and carbonic acid fractions. Any change in 'H' ion activity will be met by an adjustment in the reactions that maintain homeostasis. As long as this ratio is maintained, the blood pH will be normal. Blood pH is maintained mainly by the following buffering systems in blood:

1. Carbonic acid—bicarbonate
2. Dihydrogen phosphate—mono hydrogen phosphate
3. Protein—proteinate.
4. Hemoglobin in the red blood cells

Action of Buffer Systems:

1. The carbon dioxide formed in the metabolic reactions enters the blood (and it becomes venous in the chemical sense).
2. Small decrease in blood pH takes place by shifting the ratio of weak acid to salt in the first two buffer pairs (A and B).

   A. $CO_2+H_2O \longrightarrow H_2CO_3 + H^+ + HCO_3^-$

   B. $Na_2HPO_4 + NaH_2PO_4 + H^+ + HCO_3 \longrightarrow NaHCO_3 + 2NaH_2PO_4$

   $NaHCO_3 + H_2CO_3 + HCl$
   (Formed in metabolism)
   $\longrightarrow NaCl + 2H_2CO_3$
   Weak acid

3. When acids are produced in the metabolic reactions, these are neutralized by the first two buffer systems (A and B), and the formation of weak acid and salt does not change blood pH significantly.
4. Plasma proteins (being amphoteric) release sufficient cations to account for the carriage of about 10% of total CO_2
5. The phosphates within the red cells are responsible for about 25% of the total CO_2 carried.
6. The unique buffering role of hemoglobin accounts, for 60% of the CO_2-carrying capacity of whole blood.

The action of hemoglobin as a buffer is as follows:

The buffering capacity of hemoglobin is because, in the 'oxy' form, its nature is acidic (and hence it donates ''H' ions, and in the reduced deoxygenated form, it is less acidic than oxy-hemoglobin (and hence it accepts 'H' ions). In this regard, hemoglobin functions as follows:

1. In the tissues, oxyhemoglobin dissociates.
2. Oxygen is taken up by the cells for metabolic reactions
3. CO_2 formed in metabolism dissolves in water.

   $H_2O + CO_2 \longrightarrow H_2CO_3$

   $H_2CO_3 \xrightarrow{\text{Dissociates}} HCO_3^- + H^+$

4. HCO_3^- forms bicarbonate ($NaHCO_3$) by combining with cations such as Na^+.
5. The reduced hemoglobin accepts H^+ ions.
6. In the lungs, when the blood returns for purification, reduced hemoglobin combines with oxygen and forms oxyhemoglobin and hence releases 'H' ions.
7. 'H' ions combine with HCO_3^- released by carbonate (due to dissociation) and forms H_2CO_3.
8. Carbonic anhydrase acts on H_2CO_3, to form CO_2 and H_2O, which are expelled out by lungs in expired air.

   $H_2CO_3 \xrightarrow{\text{Carbonic anhydrase}} H_2O + CO_2$

The carbon dioxide formed in the metabolic reactions exists in blood in three main fractions: (1) As a small amount of carbonic acid (carbon dioxide dissolved in water), (2) The carbamino-bound fraction transported in combination with protein (mainly hemoglobin) and (3) That carried as bicarbonate in combination with the cations, sodium, and potassium.

Role of Lungs in the Maintenance of Acid–base Balance

It is estimated that in 24 hours, the lungs remove the equivalent of 20–40 liters of 1N acid as carbonic acid from the circulated venous blood, with the help of the enzyme carbonic anhydrase, which converts carbonic acid into carbon dioxide and water.

Role of Kidneys in the Maintenance of Acid–base Balance

The normal kidney functions such as formation, acidification, concentration or dilution of urine, along with filtration of blood at glomeruli and selective reabsorption of essential substances, controlled reabsorption of salts and water, secretion of certain substances such as hydrogen ions, creatinine, and potassium ions.

Initially, when glomerular filtrate is formed, its pH is about 7.4 (alkaline); but the pH of freshly voided normal urine is always acidic (average about pH 6.3). This is due to the continuous excretion of hydrogen ions (by the tubules) in urine. This is a very important function of kidneys: The acidification of urine. It is extremely important in maintaining the acid–base balance of the body.

DISTURBED ACID–BASE BALANCE

Competency achievement: The student should be able to:

BI6.8: Discuss and interpret results of arterial blood gas analysis in various disorders

SAQ: What is the difference between respiratory and metabolic alterations by disturbance in acid–base balance?

Ans: Disturbances in acid–base balance, which are due to alterations in the content of H_2CO_3 in blood, are said to be respiratory in origin since the content of H_2CO_3 in the blood is under the control of the respiratory system.

Disturbances in acid–base balance, which are due to the alterations in the content of bicarbonate in the blood, are said to be metabolic in origin.

RESPIRATORY ACIDOSIS

BAQ: What is respiratory acidosis and compensation? What metabolic changes occur in respiratory acidosis?

Ans: Respiratory acidosis will occur when circumstances such as causing an accumulation of H_2CO_3 in the blood due to a decrease in the elimination of carbon dioxide through the lungs, due to lung diseases such as pneumonia, severe COVID-19, etc. that affect normal expiration of carbon dioxide and an increase in blood H_2CO_3. Accumulation of H_2CO_3 affects the normal 20:1 ratio of bicarbonate: H_2CO_3 leading to respiratory acidosis.

Compensation: In respiratory acidosis, there is the reabsorption of more bicarbonate by the renal tubules. The respiratory acidosis is then said to be compensated. This means that even though the amount in the blood is abnormal, the pH may be normal since the ratio of the two has been restored to normal 20:1. Hence in respiratory acidosis, both H_2CO_3 and bicarbonate may be increased.

SAQ: Name four diseases that lead to respiratory acidosis.

Ans: Respiratory acidosis may occur in the following diseases that impair respiration:

1. Pneumonia
2. Emphysema
3. Asthma
4. Depression of the respiratory center (as by morphine poisoning)

BAQ: Enumerate causes of respiratory acidosis.

Ans: Respiratory acidosis occurs due to decreased elimination of carbon dioxide due to lung disease. Decreased elimination of blood carbon dioxide increases the carbonic acid fraction of blood. The causes of decreased elimination of carbon dioxide can be because of the following reasons:

1. Factors that directly depress the respiratory center, such as drugs (narcotics, barbiturates), trauma of the central nervous system, degenerative disorders, tumors, infections of the central nervous system (meningitis, encephalitis), intracranial hemorrhage, etc.
2. Conditions that affect the respiratory apparatus, such as pulmonary infections, pulmonary fibrosis, chronic obstructive

pulmonary disease, laryngospasm, tumor of upper airways, chest wall diseases, pleural effusions leading to impaired lung motion, acute respiratory distress syndrome, etc.

In respiratory acidosis the normal 20:1 ratio of bicarbonate: H_2CO_3 is disturbed, and the pH of the blood will fall leading to acidosis.

SAQ: Enumerate general symptoms of respiratory acidosis.

Ans: General symptoms of respiratory acidosis may include chest pain, palpitations, headache, altered mental status such as severe anxiety due to hypoxia, decreased visual acuity, nausea, vomiting, abdominal pain, loss of appetite, muscle weakness, and bone pains.

SAQ: What are the general blood gas analysis laboratory findings in respiratory acidosis?

Ans: The general laboratory findings in respiratory acidosis by arterial blood tests are as follows: Decrease in blood pH, decrease in plasma chloride as plasma bicarbonate increases, PCO_2 increases, and potassium in plasma increases due to shift from cells to plasma in exchange of movement of H^+ ions in the cells.

RESPIRATORY ALKALOSIS

BAQ: What is respiratory alkalosis and compensation? What metabolic changes occur in respiratory alkalosis?

Ans: Respiratory alkalosis will occur when there is a decrease in the carbonic acid fraction with no corresponding change in bicarbonate. This is brought about by voluntary or forced hyperventilation, which washes out abnormally large quantities of carbon dioxide. Respiratory alkalosis occurs in clinical conditions such as encephalitis, hepatic coma, high fever, etc.

Compensation: In respiratory alkalosis, since the amount of carbon dioxide and related H_2CO_3 fraction decrease due to hyperventilation, bicarbonate is increased in the blood. To compensate for this change, more bicarbonate is excreted in the urine by the kidneys. The respiratory alkalosis is then said to be compensated. This means that even though the amount of bicarbonate in the blood is on the increase, the pH may be normal; since the ratio of the two has been restored to normal 20:1. Hence in respiratory alkalosis, both H_2CO_3 and bicarbonate may be decreased.

BAQ: Enumerate the causes of respiratory alkalosis.

Ans: Respiratory alkalosis occurs due to the increased elimination of carbon dioxide. The causes of the increased elimination of carbon dioxide are as follows:

1. Non-pulmonary stimulation of the respiratory center due to hysteria, anxiety, febrile state, metabolic encephalopathy, septicemia (caused by gram-negative bacteria), hypoxia, drugs (salicylates, catecholamines), hyperthyroidism, etc.
2. Pulmonary disorders such as interstitial lung disease, pulmonary emboli, and congestive heart failure.

   In respiratory alkalosis, the normal 20:1 ratio of bicarbonate: H_2CO_3 is disturbed, and the pH of the blood will rise leading to alkalosis.

SAQ: Enumerate general symptoms of respiratory alkalosis and the first line of treatment.

Ans: The general symptoms of respiratory alkalosis may include dizziness, lightheadedness, and numbing of the hands and feet.

Treatment includes breathing into a paper bag or a mask that induces rebreathing of carbon dioxide.

SAQ: What are the general blood gas analysis laboratory findings in respiratory alkalosis?

Ans: The general laboratory findings by arterial blood tests in respiratory alkalosis are as follows: Blood pH increases and PCO_2 decreases along with normal (or decreased) plasma bicarbonate fraction.

METABOLIC ACIDOSIS

LAQ: Describe metabolic acidosis under the following heads:

1. Biochemical basis
2. Compensation
3. Clinical features
4. Related diseases and diagnosis
5. First line of treatment

Ans:

1. **Biochemical basis:** Metabolic acidosis is caused by a decrease in the bicarbonate fraction, with no change or a relatively smaller change in the carbonic acid fraction. Bicarbonate fraction decreases in the buffering of excess acids produced in the following circumstances:
   a. Production of acids that exceeds the rate of elimination, e.g. excessive formation of acetoacetic acid and beta-hydroxy-butyric acid in uncontrolled diabetes mellitus, lactic acid in lactic acidosis, and excessive formation of formic acid in methanol poisoning, etc.
   b. Significant decrease in the excretion of H^+ ions in renal failure.
   c. Excessive loss of duodenal fluid, as in diarrhea.
   d. Excessive loss of bicarbonate due to decreased reabsorption of bicarbonate by renal tubules.
2. **Compensation:** Compensation will occur in metabolic acidosis by elimination of more CO_2 (hyperventilation). The CO_2 content of the plasma will be lower than normal in metabolic acidosis.
3. **Clinical features:** Mild conditions of acidosis may be accompanied by weakness, nausea, and vomiting. Most often, severe metabolic acidosis (pH <7.20) is associated with increased respiration to compensate for decreased bicarbonate ions. Deep and rapid breathing is called Kussmaul respiration.
4. **Related diseases and diagnosis:** Metabolic acidosis can be diagnosed based on arterial blood gas analysis. The general laboratory findings in metabolic acidosis are as follows: Blood pH decreases, plasma bicarbonate decreases, and PCO_2 may be normal or decreased, decrease in plasma electrolytes (sodium, potassium, and chloride).
5. **First line of treatment:** Intravenous (IV) standard bicarbonate solution to immediately compensate for the loss of blood bicarbonate, by monitoring levels of serum electrolytes, and serum ionized calcium.

SAQ: Name four diseases that lead to metabolic acidosis.

Ans: Metabolic acidosis may occur in the following diseases and clinical conditions:

1. Uncontrolled diabetes with ketosis
2. Severe renal disease
3. Prolonged diarrhea (excessive loss of intestinal fluid)
4. Poisoning by an acid salt

BAQ: Enumerate causes of metabolic acidosis.

Ans: Metabolic acidosis is caused by a decrease in the bicarbonate fraction, with no change or a relatively smaller change in the carbonic acid fraction. Bicarbonate fraction decreases in the buffering of excess acids produced in the following circumstances:

1. Production of acids that exceeds the rate of elimination, e. g. excessive formation of acetoacetic acid and beta-hydroxy-butyric acid in uncontrolled diabetes mellitus, lactic acid in lactic acidosis, and excessive formation of formic acid in methanol poisoning, etc.
2. Significant decrease in the excretion of H^+ ions in renal failure.
3. Excessive loss of duodenal fluid, as in diarrhea
4. Excessive loss of bicarbonate due to decreased reabsorption of bicarbonate by renal tubules.

SAQ: Enumerate symptoms of metabolic acidosis.

Ans: The predominant symptoms of acidosis are sometimes difficult to distinguish from symptoms of an underlying disease or disorder. Mild conditions of acidosis may be asymptomatic or may be accompanied by weakness or listlessness, nausea, and vomiting.

Most often, severe metabolic acidosis (pH < 7.20) is associated with increased respiration to compensate for decreased bicarbonate ions. Deep and rapid breathing is called Kussmaul respiration, which is classically associated with diabetic ketoacidosis. Rapid, deep breaths increase the amount of carbon dioxide exhaled, thus lowering the serum carbon dioxide levels, resulting in some degree of compensation.

SAQ: What is the importance of the determination of anion gap in metabolic acidosis?

Ans: The presence of an elevated anion gap is often an indication of metabolic acidosis. A high anion gap is observed in uncontrolled severe diabetes, starvation, methanol and ethanol poisoning, lactic acid acidosis, etc.

However, metabolic acidosis is also observed when the anion gap is normal in the following clinical conditions: Gastrointestinal fluid loss and renal tubular acidosis.

SAQ: Enumerate general laboratory test findings in metabolic acidosis.

Ans: General laboratory findings in metabolic acidosis are as follows: Decrease in blood pH, plasma bicarbonate decreases, and PCO_2 may be normal or decreased. Decrease in plasma electrolytes (sodium, potassium, and chloride).

In renal failure, plasma inorganic phosphorus increases. Chronic metabolic acidosis increases the mobilization of calcium from bone, and decreased plasma pH increases the dissociation of plasma protein-bound calcium, which is lost in the urine with decreased reabsorption by renal tubules.

METABOLIC ALKALOSIS

LAQ: Describe metabolic alkalosis under the following heads:

1. Biochemical basis
2. Compensation
3. Reasons
4. Clinical features
5. Diagnosis and
6. First-line of treatment.

Ans:

1. **Biochemical basis:** Metabolic alkalosis occurs when there is an increase in the bicarbonate fraction with either no change or a relatively smaller change in the carbonic acid fraction. Alkalosis occurs when excess base is added to the system or in decreased elimination of base and when acid-rich fluids are lost. Any one of these clinical conditions can lead to primary bicarbonate excess.
2. **Compensation:** In metabolic alkalosis, compensation occurs by retention of CO_2 by depressed respiration activity of the lungs. The CO_2 content of the plasma will be higher than normal in metabolic alkalosis.
3. **Reasons:** Metabolic alkalosis may occur due to the following various reasons:
   1. Ingestion of alkalies such as bicarbonate or citrate (in massive blood transfusion).
   2. Pyloric stenosis leading to prolonged vomiting due to intestinal obstruction, leading to loss of hydrochloric acid and with it, chloride ions.

   In these clinical conditions, bicarbonate fraction increases in blood to compensate for chloride loss leading to a change in the 20:1 ratio of bicarbonate: H_2CO_3.
4. **Clinical features:** The predominant symptoms of alkalosis are neuromuscular hyperexcitability and irritability. Metabolic alkalosis may lead to cramping, muscle weakness, and polyuria.
5. **Diagnosis:** Metabolic alkalosis can be diagnosed based on arterial blood gas analysis. Following are the general laboratory finding in metabolic alkalosis: Blood pH increases, and plasma bicarbonate increases with a decrease in serum potassium.

6. **First-line of treatment:** Intravenous saline to immediately cope with the loss of chloride ions.

SAQ: Write general laboratory findings related to blood gas analysis in metabolic alkalosis.

Ans: The general arterial blood gas reports are as follows: Blood pH increases, and plasma bicarbonate increases with a decrease in serum potassium.

SAQ: Enumerate clinical features of metabolic alkalosis.

Ans: The general clinical features of alkalosis are neuromuscular hyperexcitability and irritability. Metabolic alkalosis may lead to cramping, muscle weakness, and polyuria.

SAQ: Write two reasons that may lead to metabolic alkalosis.

Ans:

1. Ingestion of alkalies such as bicarbonate or citrate (in massive blood transfusion).
2. Pyloric stenosis leading to prolonged vomiting due to intestinal obstruction, leading to loss of hydrochloric acid and with it, chloride ions.

BAQ: Present in tabular form laboratory findings in compensated acidosis and alkalosis.

Ans:

Table 9.1: Laboratory findings in compensated acidosis and alkalosis

	Clinical condition	pH	PCO_2	Bicarbonate	PO_2
1.	Respiratory acidosis	Fall	Rise	Rise	Rise
2.	Respiratory alkalosis	Rise	Fall	Fall	Fall
3	Metabolic acidosis	Fall	Fall	Fall	Fall
4	Metabolic alkalosis	Rise	Rise	Rise	Rise

BAQ: Give normal values of parameters of arterial blood gas analysis.

Ans:	**Reference range Normal values**
Arterial blood pH	7.38–7.42
Arterial PCO_2:	35–45 mm Hg
Arterial PO_2:	95–100 mm Hg
Standard bicarbonate:	21–28 mEq/L
Base excess of blood:	2.3 to + 2.3 mEq/L

LAQ: Give information on the type of blood, anticoagulant used, and storage of specimen for blood gas analysis. Write the clinical significance of determinations of arterial blood pH, PCO_2, $PO_{2,}$ and plasma bicarbonate.

Ans: The pH of blood: The existence of uncompensated acidosis or alkalosis is most accurately determined by the determination of blood pH. For the determination of blood pH, heparinized whole arterial blood (or heparinized capillary blood) is used. Fasting specimens is not necessary. The blood pH determination is performed immediately after the collection of blood. The blood can be stored at 0–4°C for up to 2–3 hours, without any significant change in pH.

PCO_2: It is the respiratory parameter. Plasma carbonic acid cannot be determined directly, but it is determined by measuring PCO_2. The PCO_2 of arterial blood is usually directly proportional to the amount of carbon dioxide which is being produced in the body and inversely proportional to the rate of alveolar ventilation in the lungs.

PO_2: Determination of PO_2 is carried out to assess the oxygen-carrying capacity of blood hemoglobin. The decreased oxygen affinity of the hemoglobin is indicated by the elevated PO_2 values. The measurement of arterial PO_2 is also used in conjunction with that of PCO_2 in the assessment of respiratory disorders. A low PO_2 is a measure of anoxia. It may occur with a high PCO_2 when there is alveolar hyperventilation due to depression or obstruction of respiration. A low PO_2, with low PCO_2, may also be observed in pulmonary edema.

The plasma bicarbonate: is the metabolic parameter (non-respiratory parameter). It can be determined by finding out the actual bicarbonate concentration of plasma separated from blood taken anaerobically and expressed as milli-equivalents per liter. This can be calculated from the pH and PCO_2 by using the Henderson-Hasselbalch equation or from the total carbon dioxide and the PCO_2.

Base excess is the amount of acid required to titrate blood to pH 7.40 at 37°C and PCO_2 40 mmHg. The base deficit is the reverse concept.

TCO_2 is the combination of PCO_2 and dissolved carbon dioxide as carbonic acid.

BAQ: Explain the use of the Henderson-Hasselbalch equation in the determination of blood pH, H_2CO_3 (respiratory parameter), and bicarbonate (HCO_3, metabolic parameter).

Ans: The interrelation of TCO_2, bicarbonate, carbonic acid, PCO_2, and pH in the Henderson-Hasselbalch equation is as follows:

$$\text{pH} = \text{pk} + \log \frac{\text{(Conjugate base)}}{\text{(non-ionized acid)}}$$

- For blood plasma at 37°C, $\text{pH} = 6.103 + \log \dfrac{HCO_3}{H_2CO_3}$
- The concentration of **H_2CO_3** in mEq/L, can be determined from the measurement of PCO_2 and by using the following equation:

  $$H_2CO_3 = 0.0306 \times PCO_2$$

- For normal plasma, at PCO_2 of 40 mm/Hg

  $$\begin{aligned} H_2CO_3 &= 0.0306 \times 40 \\ &= 1.224 \text{ mEq/L} \end{aligned}$$

- Concentration of **bicarbonate (HCO_3)** = $TCO_2 - H_2CO_3$
- **pH** of plasma at 37°C

  $$= \frac{\log(\text{total } CO_2) - (0.0306 \times PCO_2)}{(0.0306 \times PCO_2)}$$

Multiple Choice Questions

Q1. The normal ratio between the bicarbonate and carbonic acid fractions at the pH of blood 7.4 is

A. 1: 10
B. 20: 1
C. 1: 20
D. 1: 40

Q2. What is the amount of carbonic acid removed by the lungs in 24 hours through lungs (as $H_2O + CO_2$)?

A. 1–2 liters
B. 20–40 liters
C. 500–700 ml
D. 200–300 ml

Q3. Important buffer systems of blood are

A. Bicarbonate/carbonic acid
B. Disodium hydrogen phosphate/sodium dihydrogen phosphate
C. Plasma proteins
D. All of the above

Q4. The nature of hemoglobin in oxy-form is

A. Acidic
B. Basic
C. Neutral
D. Amphoteric

Q5. Respiratory acidosis occurs due to

A. Retention of bicarbonate
B. Excessive elimination of carbon dioxide
C. Retention of carbon dioxide
D. Excessive elimination of bicarbonate

Q6. Respiratory acidosis may occur in which of the following clinical conditions?

A. Emphysema
B. Liver disease
C. Kidney disease
D. A and B

Q7. Respiratory alkalosis can occur in

A. Emphysema
B. Asthma
C. Hysterical hyperventilation
D. Bronchial obstruction

Q8. The primary event in respiratory alkalosis is

A. Rise in pH
B. Decrease in bicarbonate
C. Increase in plasma bicarbonate
D. Decrease in PCO_2

Q9. Uncontrolled diabetes with ketosis may lead to

A. Respiratory alkalosis
B. Metabolic acidosis
C. Respiratory acidosis
D. Metabolic alkalosis

Q10. Following features are found in uncompensated lactic acidosis *except*

A. Anion gap is normal
B. Bicarbonate is decreased
C. PCO_2 is normal
D. Decrease in blood pH

Q11. All of the following statements about renal tubular acidosis are correct *except*

A. Renal tubules may be unable to reabsorb bicarbonate
B. Renal tubules may be unable to secrete hydrogen ions
C. Anion gap is decreased
D. Plasma chloride is elevated

Q12. All of the following changes may occur in blood in severe diarrhea *except*

A. Increase in PCO_2
B. Decrease in sodium
C. Decrease in pH
D. Decrease in chloride

Q13. During compensation of respiratory alkalosis, all of the following changes occur *except*

A. Decreased secretion of hydrogen ions by renal tubules
B. Increase in blood bicarbonate
C. Increased excretion of bicarbonate in urine
D. Increase in blood pH

Q14. Following changes may be observed in blood in compensated respiratory acidosis

A. Increase in PCO_2
B. Increase in bicarbonate
C. increase in blood pH
D. A and B

Q15. Metabolic alkalosis can occur in

A. Severe diarrhea
B. Renal failure
C. Diabetic ketosis
D. Pyloric stenosis

Q16. Following features may be present in blood in uncompensated metabolic alkalosis *except*

A. Increased pH
B. Increased bicarbonate
C. Normal chlorides
D. Decreased bicarbonate

Q17. Blood total carbon dioxide means

A. Plasma bicarbonate
B. Dissolved CO_2
C. Plasma lactic acid
D. A and B

Q18. Decreased blood PO_2 means

A. Reduced ability of oxygen to diffuse from alveolar air into blood.
B. Reduced ability of CO_2 to diffuse from alveolar air into the blood.
C. Increased ability of CO_2 to diffuse from alveolar air into blood.
D. B or C

Q19. A 66-year-old diabetic person reported high fever, throat infection, difficulty in breathing, and blood oxygen saturation of 86% (Normal: 95–100%). What is the immediate first line of treatment?

A. Saline IV drip
B. Oxygen supply
C. Paracetamol on hyperthermia
D. A, B, and C

Q20. A 43-year-old woman reported high fever, cough, loss of smell, and acute respiratory distress syndrome (ARDS) during covid-19 pandemic. Her Blood pH was 7.28 (normal 7.38–7.42), PO_2 was 82 mmHg (normal 90–100 mmHg), PCO_2 was 65 mmHg (normal: 35–45 mmHg), and plasma bicarbonate was 24 mmol/L (normal: 22–26 mol/l). He was suffering from

A. Metabolic acidosis
B. Respiratory acidosis
C. Metabolic alkalosis
D. Respiratory alkalosis

Answers

1. B **2.** B **3.** D **4.** A **5.** C **6.** A **7.** C **8.** D **9.** B **10.** A
11. C **12.** A **13.** B **14.** D **15.** D **16.** D **17.** D **18.** A

19. D. Since his blood oxygen supply was significantly low (86%), he had difficulty in breathing probably due to severe pneumonia and fluid replacement was necessary to maintain body fluid balance in hyperthermia.

20. B. Since PCO_2 is the respiratory parameter, which is increased with plasma bicarbonate normal. Hence the clinical condition is acidosis and of respiratory type. Decreased functions of the lungs are seen from the low PO_2 values.

Case Studies

Case 1: A 67-year-old man met an accident and was admitted to the hospital with severe abdominal pain. He had a distended rigid abdomen. His arterial blood reports related to acid base balance were as follows:

	Reference range (Normal values)
Blood pH: 7.1	7.38–7.42
PCO_2: 27 mm Hg	35–45 mm Hg
PO_2: 103 mm Hg	80–100 mm Hg
Standard bicarbonate: 12.5 mmol/L	22–26 mmol/L
O_2 saturation: 94%	95–100%

1. What is the probable diagnosis?

Ans: Metabolic acidosis, since blood pH was <7.38, and a significant decrease in bicarbonate fraction, which is a metabolic parameter.

2. What is the biochemical basis for a decrease in blood pH?

Ans: A decrease in blood pH is related to an abdominal injury, leading to lactic acid acidosis due to impaired tissue perfusion, which caused inadequate oxygenation with increased anaerobic metabolism of glucose to lactic acid.

3. What is the first line of treatment?

Ans: Ineffective tissue perfusion can be a life-threatening emergency. It requires critical thinking and strict monitoring of the clinical condition by the emergency staff. Appropriate measures should be taken to normalize disturbed perfusion and improvement in blood circulation in the damaged portion of the abdomen of the patient. Blood flow and perfusion pressure at the damaged organ can be controlled by appropriate fluid replacement, which restores the following biochemical arterial blood parameters: pH, PCO_2, and PO_2.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology.

Ans: Horizontal integration with anatomy

Right distended abdomen, which leads to metabolic acidosis

Horizontal integration with physiology

Presence of lactic acid acidosis and a significant decrease in blood pH and bicarbonate.

BAQ: Show vertical integration of symptoms and test reports of Case 1 with general medicine, pharmacology and pathology.

Ans: Vertical integration with pharmacology

Study on the use of appropriate tissue conditioning and vasoactive drugs.

Vertical integration with general medicine

Study of trauma-related metabolic acidosis.

Vertical integration with pathology

Study of the basic causes of metabolic acidosis, pathophysiology and prognosis.

Case 2: A 48-year-old woman was admitted to the hospital with severe pneumonia. Her arterial blood reports related to acid–base balance were as follows:

	Reference range (Normal values)
Blood pH: 7.2	7.38–7.42
PCO_2: 62 mm Hg	35–45 mm Hg
PO_2: 74 mm Hg	80–100 mm Hg
Standard bicarbonate: 28 mmol/L	22–26 mmol/L
O_2 saturation: 87%	95–100%

1. What is the probable diagnosis?

Ans: Respiratory acidosis, since blood pH was <7.38, the significant increase in PCO_2, which is a respiratory parameter, and decrease in PO_2 indicate dysfunction of lungs related to severe pneumonia.

2. What is the biochemical basis for the decrease in blood pH?

Ans. Decrease in normal expiration of carbon dioxide, lead to disturbance in the 20:1 ratio of bicarbonate: H_2CO_3 and decrease in blood pH.

3. What is the first line of treatment?

Ans:

A. Use of appropriate anti-microbial drugs, if microbial infection is suspected.

B. Use of bronchodilator to clear airway obstruction and appropriate use of corticosteroids to decrease cytokines related to inflammation of airways.

C. Non-invasive positive-pressure ventilation for the supply of oxygen to restore blood oxygen level.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology

Ans: Horizontal integration with anatomy

Inflammation of lung tissue, and alveoli due to pneumonia.

Horizontal integration with physiology

Decreased rate of carbon dioxide expiration and low circulation of blood oxygen.

BAQ: Show vertical integration of symptoms and test reports of Case 2 with general medicine, pharmacology and pathology.

Ans: Vertical integration with pharmacology

Study of appropriate drugs to treat lung infection and clear airway passage

Vertical integration with general medicine

Study of respiratory acidosis related to pneumonia.

Vertical integration with pathology

Study of causes of respiratory acidosis related to pneumonia, pathophysiology and prognosis.

Case 3: A 36-year-old man was admitted to the hospital with a history of prolonged vomiting. He looked dehydrated. His respiration was shallow. His blood reports related to acid–base balance were as follows:

	Reference range (Normal values)
Blood pH: 7.50	7.38–7.42
PCO_2: 53 mm Hg	35–45 mm Hg
PO_2: 75 mm Hg	80–100 mm Hg
Standard bicarbonate: 44 mmol/L	22–26 mmol/L
O_2 saturation: 87%	95–100%

1. What is the probable diagnosis?

Ans: Metabolic alkalosis, since blood pH was >7.42. and the significant increase in plasma bicarbonate, which is a metabolic parameter.

2. What is the biochemical basis for the increase in blood pH?

Ans. Loss of gastric acid due to prolonged vomiting, lead to an equivalent increase in serum bicarbonate. Normal 20:1 ratio of bicarbonate: H_2CO_3 changed due to an increase in plasma bicarbonate.

3. What is the probable line of treatment?

Ans: Prolonged vomiting may be due to severe gastrointestinal inflammation due to infection, parasitic infection (roundworm infestation), or gastrointestinal obstruction. According to the cause of metabolic alkalosis, this case is treated by:

A. Use of appropriate anti-microbial drugs, if microbial infection or parasitic infestation is suspected.

B. Surgical intervention by removal of the obstruction in the intestine.

C. Use of saline drips to restore chloride loss.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology.

Ans: Horizontal integration with anatomy

Affected gastrointestinal tract, due to obstruction or increased inflammation.

Horizontal integration with physiology

Significant decrease in plasma chlorides due to prolonged vomiting.

BAQ: Show vertical integration of symptoms and test reports of Case 3 with general medicine, pharmacology, surgical medicine and pathology.

Ans: Vertical integration with general medicine

Study of metabolic alkalosis related intestinal infection and obstruction.

Vertical integration with pharmacology
Study on the use of appropriate drugs to treat infection and drugs to compensate for serum chloride loss.

Vertical integration with surgical medicine
Consideration of surgical methods for the removal of obstruction in the gastrointestinal tract to the food passage.

Vertical integration with pathology
Study of causes of metabolic alkalosis related intestinal infection and obstruction, pathophysiology and prognosis.

Case 4: A 45-year-old woman was admitted to the hospital after she experienced muscular weakness and cramps. She showed signs of hyperventilation. It was found out that she had taken an overdose of aspirin.

	Reference range (Normal values)
Blood pH: 7.54	7.38–7.42
PCO_2: 24 mm Hg	35–45 mm Hg
PO_2: 70 mm Hg	80–100 mm Hg
Standard bicarbonate: 26 mmol/L	22–26 mmol/L

1. What is the probable diagnosis?
Ans: Respiratory alkalosis, since blood pH was >7.38. A significant decrease in PCO_2 which is a respiratory parameter, indicates respiratory alkalosis.

2. What is the biochemical basis for an increase in blood pH?
Ans: Loss of carbon dioxide (PCO_2), due to hyperventilation. Normal 20:1 ratio of bicarbonate: H_2CO_3 changed due to a decrease in blood PCO_2.

3. What is the first line of treatment?
Ans: Stoppage of the drug (aspirin) intake that leads to the increase in blood pH and intake of adequate fluids to excrete excess aspirin metabolites in blood circulation.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology.

Ans: Horizontal integration with anatomy
Hyperactive lungs (leading to hyperventilation).

Horizontal integration with physiology
Significant decrease in blood PCO_2

BAQ: Show vertical integration of symptoms and test reports of this patient with general medicine.

Ans: Vertical integration with general medicine
Study of drug-induced respiratory alkalosis

Case 5: A 58-year-old man was admitted to the hospital with a history of renal disease. He presented with generalized edema, loss of weight, and oliguria. His acid–base related blood reports were as follows:

	Reference range (Normal values)
Blood pH: 7.25	7.38–7.42
PCO_2: 36 mm Hg	35–45 mm Hg
PO_2: 90 mm Hg	80–100 mm Hg
Standard bicarbonate: 17 mmol/L	22–26 mmol/L
O_2 saturation: 94%	95–100%

1. What is the probable diagnosis?
Ans: Metabolic acidosis, since blood pH was 7.30, and a significant decrease in serum bicarbonate, which is a metabolic parameter. Decrease in plasma bicarbonate, with no significant change in PCO_2 leads to acidosis.

2. What is the biochemical basis for a decrease in blood pH?
Ans: The patient was suffering from renal disease. One very important function of kidneys is the acidification of urine, which means the excretion of excess acids produced during metabolic reactions in urine for the maintenance of homeostasis. Acidification of urine is affected in renal (kidney) disease. Accumulated organic acids in the blood decrease the pH of the blood.

3. What is the probable line of treatment?
Ans. It is necessary to treat the basic cause of renal disease. Restoration of kidney

functions by appropriate treatment and appropriate management of the case to maintain homeostasis.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology.

Ans: Horizontal integration with anatomy

Diseased dysfunctional kidneys.

Horizontal integration with physiology

Affected acidification of urine, due to kidney dysfunction.

BAQ: Show vertical integration of symptoms and test reports of Case 5 with general medicine and pathology.

Ans: Vertical integration with general medicine

Study of kidney disease-related metabolic acidosis.

Vertical integration with pathology

Study of causes of kidney disease-related metabolic acidosis, pathophysiology and prognosis.

Case 6: A 72-year-old man presented with a history of weight loss, blurred vision, increased rate of urination, dry mouth, and uncontrolled diabetes mellitus. A random chemical examination of urine indicated the presence of a high percentage of glucose and ketone bodies. His acid–base related blood reports were as follows:

	Reference range Normal values
Blood pH: 7.30	7.38–7.42
PCO_2: 45 mm Hg	35–45 mm Hg
PO_2: 63 mm Hg	80–100 mm Hg
Standard bicarbonate: 18 mmol/L	22–26 mmol/L
Urine ketone bodies: Present^{+++}	Absent

1. What is the probable diagnosis?

Ans: Metabolic acidosis, since blood pH was <7.38, and a significant decrease in serum Bicarbonate, which is a metabolic parameter.

2. What is the biochemical basis for a decrease in blood pH?

Ans: The patient was suffering from uncontrolled diabetes. Since body cells were unable to use glucose for energy (in the form of ATP molecules), free fatty acids (FFA) were used. The intermediate product of FFA metabolism is acetyl-CoA, which is partly converted to ketone bodies: Acetone, acetoacetic acid, and beta-hydroxybutyric acid. These ketone bodies being acidic, decrease the blood pH.

3. What is the probable line of treatment?

Ans: Urgent diagnosis of the type of diabetes mellitus and then effective treatment to restore normal blood glucose metabolism.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy and physiology

Ans: Horizontal integration with anatomy

Affected body weight, eyes and kidneys in uncontrolled diabetes mellitus.

Horizontal integration with physiology

Excretion of excessive ketone bodies. Increase in the frequency of urination.

BAQ: Show vertical integration of symptoms and test reports of case 6 with general medicine, pharmacology, and pathology.

Ans: Vertical integration with general medicine

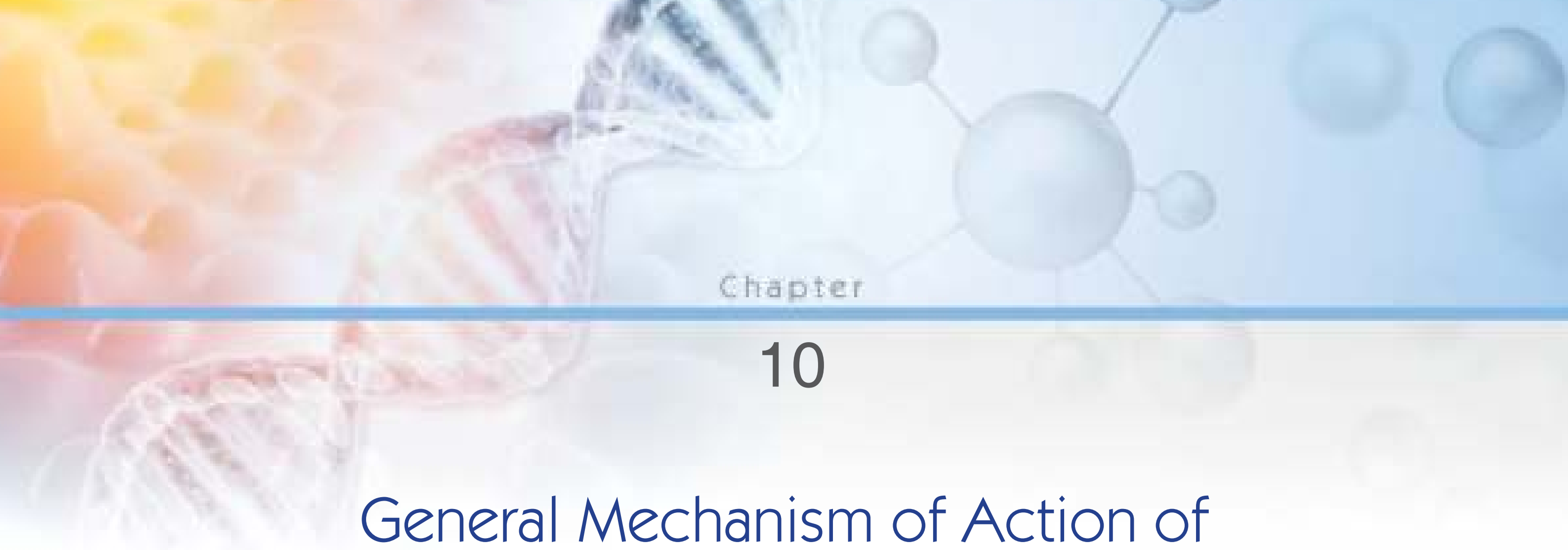
Study of diabetes-related metabolic acidosis.

Vertical integration with pharmacology

Study of an appropriate drug on diabetes mellitus related acidosis and ketosis.

Vertical integration with pathology

Study of causes of diabetes-related metabolic acidosis, pathophysiology and prognosis.



Chapter

10

General Mechanism of Action of Hormones

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

INTRODUCTION

A hormone is a chemical substance released by a cell in one part of the body that sends out messages which act on cells in other parts of the organism. A very small amount of hormone is required to act on a cell and alter cell metabolism. Most hormones initiate a cellular response by initially combining with either a specific intracellular or cell membrane-associated receptor protein. A cell may have several different receptors that recognize the same hormone and activate different signal transduction pathways.

SAQ: What are hormones?

Ans: Hormones are secreted by endocrine or ductless glands. They catalyze and control diverse metabolic processes.

BAQ: What is the similarity and difference between hormones with enzymes?

Ans: Hormones resemble enzymes in the following two ways:

1. They act as catalysts.
2. They are needed in small amounts and are not used up in the reaction.

The differences between enzymes and hormones are described below:

1. Enzymes act on substrates at the cellular level, where these are synthesized. However, hormones are synthesized at the endocrine gland and act on the cells of a target organ.
2. All enzymes are protein in nature. However, all hormones are not protein in nature, only some hormones are protein in nature.

SAQ: Enumerate names of signaling molecules apart from the hormones.

Ans: Other signaling molecules apart from hormones are growth factors, cytokines, interleukins, and specific metabolites which use similar general pathways and signal transduction pathways as hormones.

BAQ: Write five factors that regulate the action of the hormones.

Ans: At a target organ, the action of hormones is regulated by 5 factors:

1. Rate of synthesis and secretion of the stored hormone from the endocrine gland.
2. Specific transport systems in plasma (in some cases).
3. Conversion to the more active form (in some cases).
4. Hormone-specific receptors in target cells.
5. Ultimate degradation of the hormone mainly by the liver or kidneys.

BAQ: Write a note on the general mechanisms of the actions of hormones.

Ans: The following points describe the general mechanism of actions of hormones:

1. Many hormones stimulate RNA production in the target cell nucleus. This action increases the synthesis of a specific enzyme or group of enzymes, catalyzing a specific metabolic pathway.
2. Hormonal action may stimulate the translation of information carried by the messenger RNA on the ribosomes to the production of the enzyme protein.
3. Many enzymes are activated by the direct action of hormones.
4. Many hormones activate different membrane enzyme systems by direct binding to specific receptors on the cell membrane. These hormones are specifically involved in the transport of a variety of substances across cell membranes, such as glucose, amino acids, nucleotides, and various cations.
5. Many hormones act by changing the level of cyclic AMP.

   Cyclic AMP is formed by the following action of the enzyme adenyl cyclase on ATP in the presence of magnesium ions:

$$\text{ATP} \xrightarrow[\text{Mg++}]{\text{Adenyl cyclase}} \text{Cyclic-3, 5-AMP}$$

Q: Enumerate the various functions of cyclic AMP.

Ans: The following are the various functions of cyclic AMP:

1. Activation of a large variety of phosphokinase enzymes.
2. Activation of lipolysis in adipose tissue.
3. Stimulation of glycogenolysis and inhibition of gluconeogenesis.
4. Action as a second messenger for other hormones.

SAQ: What are paracrine and autocrine hormones? Give two examples each.

Ans: Paracrine hormones: They act on the cells adjacent or close to the cells from where they are released, e.g. prostaglandins and polypeptide growth factors. The polypeptide growth factors are involved in the control of cell proliferation.

Autocrine hormones: They act on the same cells where they are synthesized, e.g. interleukin-1 and interleukin-2 produced by T-cells are responsible for the stimulation of their proliferation.

CLASSIFICATION OF HORMONES

LAQ: Write a note on the classification of hormones.

Ans: Hormones can be classified as follows:

1. Classification based on the chemical nature of the hormone:

   These hormones can be classified into the following three groups:

   A. Protein or peptide hormones, e.g. insulin, antidiuretic hormone (ADH), oxytocin, glucagon, etc.

   B. Steroid hormones, e.g. sex hormones, glucocorticoids, mineralocorticoids, etc.

   C. Amino acid derivatives, e.g. thyroxine (T4), triiodothyronine (T3), epinephrine, norepinephrine, etc.

2. Classification based on the mechanism of action exerted by a hormone:

   Hormones can also be classified into the following two broad groups based on the location of receptors to which they bind and the signals used to mediate their action. The following are two groups related to this category:

   A. *Group 1 hormones:* These hormones are lipid soluble and can pass through the cell membrane and bind to intracellular receptors to form receptor-hormone complexes, through which their biochemical functions are mediated. Except for T_3 and T_4, group 1 hormones are derivatives of cholesterol, e.g. estrogens, androgens, glucocorticoids, etc. These hormones act through the intracellular receptors located either in the cytosol or the nucleus. The hormone receptor complex binds to specific regions on the DNA (hormone-responsive element) and causes increased expression of specific genes. The outcome is the production of specific proteins in response to hormonal action.

B. *Group 2 hormones:* These hormones are water-soluble enzymes and are not able to pass through the cell membrane. These hormones bind to cell surface (plasma membrane) receptors and stimulate the release of second messengers (certain molecules). These messengers perform biochemical functions. These hormones are hydrophilic and usually transported in free form, and possess short half-lives. These hormones can be further divided into the following three categories based on the chemical nature of the second messengers:

1. The hormones such as ACTH, FSH, LH, PTH, glucagon and calcitonin. These stimulate the release of second messengers-cAMP.
2. The hormones such as TRH, gastrin, etc. stimulate the release of phosphatidyl inositol/calcium as a second messenger.
3. The hormones such as growth hormones, insulin, oxytocin, prolactin, etc. Two molecules have been proposed as second messengers transducing the insulin signal into the target cell. One is a phospho-oligosaccharides/inositol phosphoglycan and the other is diacylglycerol, both deriving from the same plasma membrane glycolipid.

SIGNAL TRANSDUCTION BY HORMONES

BAQ: Write a note on signal transduction by hormones.

Ans: Signal transduction means the process by which a cell responds to substances outside the cell through signaling molecules found on the surface of and inside the cell. Signal transduction by hormones takes place as follows:

- The stimulus can be a threat or challenge to a single cell, organ, or organism. Recognition of the stimulus is the first step in the adaptive response. This generally involves the special senses (touch, sight, smell, pain, hearing) and the nervous system.
- At the cellular level, recognition of the stimulus involves temperature, pH, nutrient supply, oxygen tension, osmolarity, and harmful metabolites.
- Appropriate response results in the release of the appropriate release of hormones that generate the necessary adaptive response in the form of gene transcription, specific protein synthesis, protein translocation, protein modification, and protein transportation through selective channels to required sites.
- Lipid-soluble hormones interact with an intracellular receptor, and water-soluble hormones interact with receptor recognition sites located on the extracellular surface of the plasma membrane of a target cell.
- Lipid soluble hormones are lipophilic and diffuse through the plasma membrane, and interact specifically with the receptors located in the cytoplasm or nucleus.
- Water soluble hormones do not have transport proteins. Hence initiate a response by binding to a receptor located in the plasma membrane. They use an intracellular signaling mechanism by using cAMP. Many water-soluble hormones bind to receptors that couple to effectors through a GTP-binding protein.

LAQ: Describe the mechanism of action of hormones.

Ans:

1. Lipid soluble hormones which are derivatives of cholesterol, e.g. estrogens, androgens, glucocorticoids, etc. can pass through the cell membrane and bind to intracellular receptors to form receptor-hormone complexes, through which their biochemical functions are mediated (Fig. 10.1a). These hormones act through the intracellular receptors located either in the cytosol or the nucleus. The hormone receptor complex binds to specific regions on the DNA (hormone-responsive element) and causes increased expression of specific genes. The outcome is the production of specific proteins in response to hormonal action.
2. Hormones such as adrenocorticotropic hormone (ACTH), antidiuretic hormone (ADH), thyroid stimulating hormone, gonadotropins, parathyroid hormone, glucagon, FSH, hCG, etc. bind to the cell membrane. These hormones use cAMP as a secondary messenger to initiate specific actions.

Example (Fig. 10.1b): Use of cAMP as a secondary messenger.

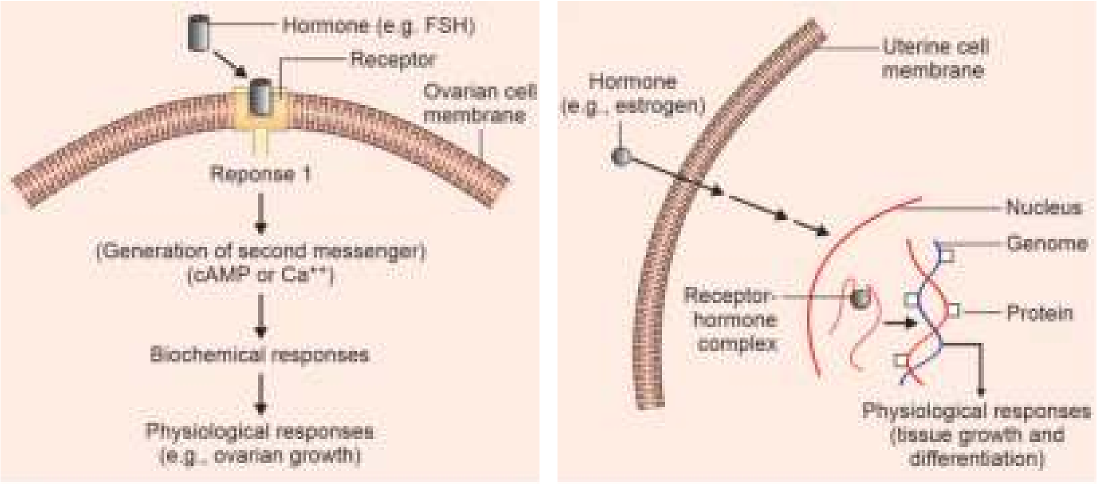
A regulatory G (G for GTP-binding) protein is associated with the cytoplasmic face of the receptor protein, which has three subunits, alpha, beta, and gamma. The alpha subunit has a site on it that can bind to either GTP or GDP. When the site on the alpha unit is occupied by GTP, initiation of cellular chemical reactions takes place. When the site is occupied by GDP, no reaction is initiated.

When a hormone, such as epinephrine, binds to a receptor (beta-adrenergic receptor) on a cell surface (of a smooth muscle), it leads to the following reactions:

A. Binding of the hormone on the specific receptor. The alpha subunit of the G complex gets detached and becomes activated.

B. Alpha subunit-GTP complex activates the adenyl cyclase enzyme, which is attached to the inner wall of the cell membrane. Adenyl cyclase acts on ATP and cyclic AMP is produced.

C. cAMP activates protein kinase A. cAMP binds to inactive protein kinase


Hormone (e.g. FSH)
Receptor
Ovarian cell membrane
Reponse 1
(Generation of second messenger) (cAMP or Ca++)
Biochemical responses
Physiological responses (e.g., ovarian growth)
Uterine cell membrane
Hormone (e.g., estrogen)
Nucleus
Genome
Protein
Receptor-hormone complex
Physiological responses (tissue growth and differentiation)

(a) Protein hormone (b) Steroid hormone

Fig.10.1: Line diagram of hormone action by protein hormone and steroid hormone

and causes the dissociation of R and C subunits of protein kinase.

D. The active subunit 'C' catalyzes the phosphorylation of protein (transfer of phosphate group to serine and threonine residues). The phosphoprotein ultimately causes the biochemical response.

E. The active form of the alpha subunit then acts on GTP to produce GDP. Once GDP is formed alpha subunit becomes inactivated since it gets again attached to the G protein.

F. Downregulation of hormonal action takes place when the hormone unbinds to the receptor. GTP is converted to GDP in the G complex, and the adenyl cyclase enzyme gets inactivated.

G. Cyclic AMP is converted to AMP by the action of the enzyme phosphodiesterase (Fig. 10.2).

3. The hormones TRH, gastrin, etc. that stimulate the release of phosphatidyl inositol/calcium, use these as a second messenger. These hormones act as follows:

Use of phosphatidyl inositol/calcium as a secondary messenger:

A. The hormones bind to specific external cell receptors and bind to the G protein which exchanges its GDP for GTP.

B. The G protein–GTP complex migrates inside the cell and activates a membrane-bound enzyme, phospholipase C (PLC).

C. PLC cleaves phosphatidyl inositol 4,5, biphosphate (PiP2) to yield 1,2, diacylglycerol, and inositol 1,4,5 triphosphate (InsP3).

D. The function of InsP3 is to increase intracellular calcium by releasing calcium ions from the lumen of the endoplasmic reticulum. These calcium ions contribute to a variety of biochemical processes (through the mediation of calmodulin). InsP3 is a short-lived chemical messenger.

E. The G protein hywdrolyses GTP to GDP and inorganic phosphorus and becomes inactivated.

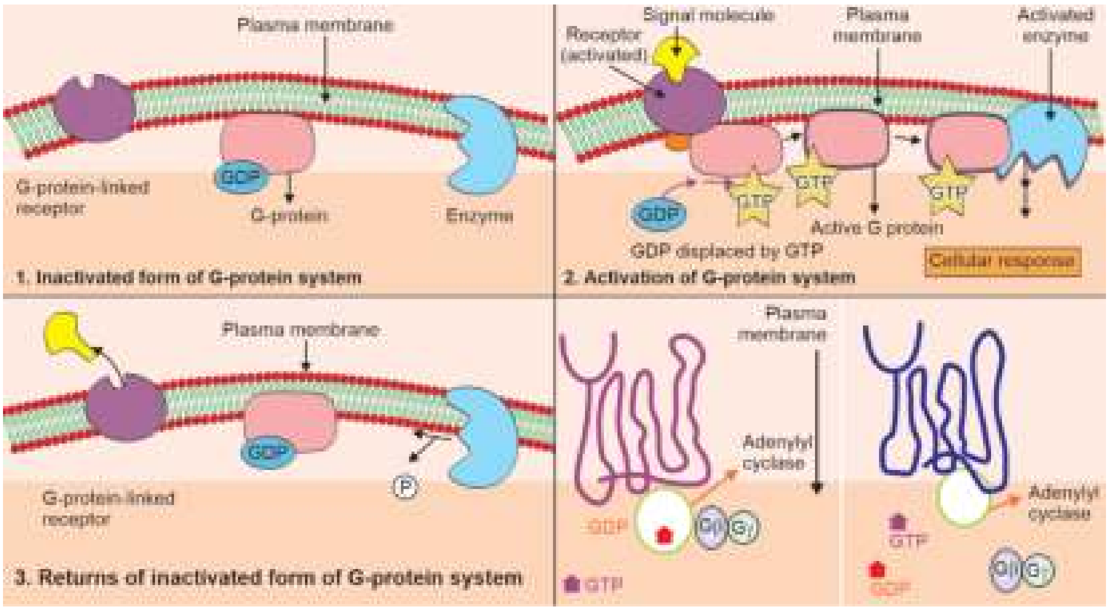

Plasma membrane
GDP
G-protein-linked receptor
G-protein
Enzyme
1. Inactivated form of G-protein system
Signal molecule
Receptor (activated)
Plasma membrane
Activated enzyme
GTP
GTP
GTP
GDP
Active G protein
GDP displaced by GTP
Cellular response
2. Activation of G-protein system
Plasma membrane
GDP
P
G-protein-linked receptor
3. Returns of inactivated form of G-protein system
Plasma membrane
Adenylyl cyclase
GDP
Gi
G
GTP
Adenylyl cyclase
GTP
Gi
G
GDP

Fig. 10.2: G-protein actions

The action of 1,2, diacylglycerol (DG): The DG being nonpolar is retained in the membrane, where it functions as a second messenger. It activates the membrane-bound enzyme-protein kinase C in the presence of calcium ions. The calcium ions are obtained from the endoplasmic reticulum by stimulating InsP3. The enzyme protein kinase C and calcium-dependent calmodulin kinase bring about protein phosphorylation.

Use of the phospho-oligosaccharides/inositol phosphoglycan: The hormones such as growth hormones, insulin, oxytocin, prolactin, etc., use the following two molecules as second messengers: One is a phospho-oligosaccharides/inositol phosphoglycan and the other is diacylglycerol (DG), both deriving from the same plasma membrane glycolipid.

The phospho-oligosaccharide/inositol phosphoglycan is derived from plasma membrane glycolipid mediated by insulin attachment. The phospho-oligosaccharide mediates many metabolic effects of insulin through control of the phosphorylation state of important regulatory metabolic enzymes.

Example: Action of insulin

A. Insulin extracellularly interacts with the alpha subunit of the insulin receptor. This interaction leads to conformational changes in the insulin-receptor complex, leading to tyrosine kinase phosphorylation of insulin receptor substrates and subsequent activation of phosphatidylinositol-3-kinase.

B. These events cause the translocation of the GLUT-4 transporter from intracellular to extracellular onto the plasma membrane of skeletal muscle cells. The rate of exocytosis of GLUT-4 vesicles increases due to the action of insulin's actions or due to exercise.

Thus, by increasing the presence of GLUT-4 on the plasma membrane, insulin facilitates glucose entry into skeletal muscle cells for metabolism into glycogen.

Q: Why exercises are advised for diabetic persons and normal individuals concerning maintaining good blood glucose control?

Ans: Exercises increase GLUT-4 transporters, responsible for the transportation of glucose to skeletal tissues. Increased utilization of glucose by skeletal muscle cells maintains normal blood glucose levels with adequate ATP molecules for cellular and muscular activities.

Q: Why a person suffering from cholera infection suffers from severe diarrhea?

Ans: Gut mucosal cells secrete sodium ions into the intestinal lumen, and this activity is stimulated by cAMP. In cholera infection, the cholera toxin inactivates the GTPase activity of the G protein alpha unit. Thus, the hormone stimulus activation of adenylate cyclase does not get terminated and remains continuous. With the result prolonged cAMP production results in a massive loss of sodium ions, accompanied by water molecules. This leads to severe diarrhea and possible death from fluid and electrolyte loss, if not treated in appropriate time.

Q: Explain the action of the drug trinitroglycerine in the treatment of angina pectoris.

Ans: Trinitroglycerine is a drug that produces nitric oxide (NO) gas. In the cytosol, guanylate cyclase is present. It has a heme molecule as its prosthetic group. The heme group binds to nitric oxide (NO), which is produced from the arginine guanidine group by nitric oxide synthase. The NO diffuses into the smooth muscle of blood vessels, causing cGMP production. cGMP causes muscle relaxation and vessel dilatation. It is used in the treatment of angina pectoris (chest pain due to reduced blood flow to the heart), since the production of NO leads to the relaxation of blood vessels and reduces the workload on heart muscles and reduction in the chest pain.

Multiple Choice Questions

Q1. In the cAMP pathway, the G protein stimulates

A. Phospholipase C
B. Adenylyl cyclase
C. The endoplasmic reticulum
D. Calmodulin

Q2. In most cases protein kinases

A. Hydrolyze proteins
B. Polymerize amino acids
C. Stimulate adenylyl cyclase
D. Add phosphate groups to proteins

Q3. The receptor for nitric oxide (NO) is

A. Intercellular
B. Intracellular
C. Extracellular
D. Unicellular

Q4. Which one of the following is a common second messenger?

A. cAMP
B. cGTP
C. ATP
D. cRNA

Q5. Paracrine signals

A. Are long-lived with widespread effects
B. Are short-lived but with widespread effects due to cascades
C. Are long-lived, but are acting locally
D. Are short-lived with local effects

Q6. Which of these interact with intracellular receptors?

A. Progesterone
B. Vitamin D
C. Cortisol
D. All of the above

Q7. Concerning the plasma membrane, most enzymatic receptors

A. Function like enzymes
B. Function like receptor
C. Function like hormones
D. Both A and B

Q8. Transmembrane proteins

A. Go from one side of a membrane through the other side of the membrane
B. Deny or permit the transport of specific substances across the biological membrane
C. Perform catalytic functions
D. Both A and B

Q9. Binding of epinephrine to a G protein-linked receptor causes adenylyl cyclase to produce large amounts of

A. G protein
B. Phospholipase C
C. Inositol triphosphate
D. cAMP

Q10. Which of the following hormones that act on the same cells, where they are secreted?

A. Paracrine
B. Autocrine
C. Merocrine
D. Holocrine

Q11. The signaling molecules that travel the farthest are

A. Endocrine
B. Paracrine
C. Neurotransmitters
D. B and C

Q12. When a signal molecule arrives at a G protein-linked receptor, the G protein

A. Binds with a Ca^{++}
B. Becomes inactivated
C. Becomes activated
D. Binds with calmodulin

Q13. Cells interact through the actions of chemical signals which either bind to receptors on the membrane or

A. Pass through the plasma membrane
B. Trigger responses from inside the cell
C. Bind to intracellular receptors
D. All of the above

Q14. Hormones are relatively long-lived signals that travel throughout the body. This type of signaling is called

A. Paracrine signaling
B. Synaptic signaling
C. Autocrine signaling
D. Endocrine signaling

Q15. All of the following statements apply to G proteins *except*

A. G proteins transmit a signal from the cell surface to the interior of the cell
B. All G proteins have a similar structure
C. G proteins do not use secondary messengers but transmit the signal directly into the nucleus
D. G proteins act to amplify the signal creating a cascade response in the cell

Q16. Beta-blockers are

A. Beta-adrenergic receptor antagonists
B. Diminish effects of epinephrine and other stress hormones
C. Used in the management of cardiac arrhythmia
D. All of the above

Q17. All these hormones that stimulate the release of second messengers-cAMP, *except*

A. TRH B. ACTH
C. FSH D. LH

Q18. The hormone that stimulates the release of phosphatidyl inositol/calcium as a second messenger

A. Gastrin B. ACTH
C. FSH D. A and B

Q19. The hormone that uses phospho-oligosaccharides/inositol phosphoglycan as a second messenger

A. LH
B. Insulin
C. Estrogens
D. TRH

Q20. Signal transduction means the process by which a cell responds

A. To substances outside the cell
B. Through signaling molecules found on the surface of the cell
C. Through signaling molecules found inside the cell
D. A, B, and C

Answers

1. B **2.** D **3.** B **4.** A **5.** D **6.** D **7.** D **8.** D **9.** D **10.** B
11. A **12.** C **13.** C **14.** D **15.** C **16.** D **17.** A **18.** A **19.** B **20.** D

Chapter

11

Vitamins

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

The term "vitamin" was formed from *vita* (the Latin word *vita* means life) and amine (an organic compound containing amino group), meaning amine of life. The vitamin is an essential organic compound required in small amounts for normal growth and activities. Vitamin cannot be synthesized by an organism, although some vitamins such as niacin can be synthesized from tryptophan and normal bacterial flora is responsible for synthesizing vitamin D_3.

Deficiencies of vitamins are classified as either primary or secondary. When an organism does not get sufficient vitamins in food, a primary deficiency occurs. A secondary deficiency may be due to an underlying disorder that prevents or limits the absorption of vitamins.

Competency achievement: The student should be able to:

BI6.5: Describe the biochemical role of vitamins in the body and explain the manifestations of their deficiency

LAQ: Describe the biochemical role of vitamins in the body and explain the manifestations of their deficiency.

Ans: A vitamin is an organic compound required as a nutrient in small amounts by an organism. A vitamin cannot be synthesized in sufficient quantities by an organism and must be obtained from the diet. Presently, thirteen vitamins are universally recognized, and these are classified according to their biological and chemical activity and not by their structure. In humans, vitamins are classified as either water-soluble or fat-soluble. In humans, there are four fat-soluble (A, D, E, and K) and nine water-soluble vitamins (eight B vitamins and vitamin C).

Vitamins have diverse biochemical functions. Some vitamins like vitamin D have hormone-like functions as regulators of mineral metabolism. Some forms of vitamin A regulate the growth and differentiation of cells and tissues. Other vitamins like E and C act as antioxidants. Similarly, B complex vitamins function as precursors for enzyme cofactors that help enzymes in their work as catalysts in several metabolic reactions.

Vitamins were obtained only through food intake and changes in diet until mid-1930. Since the middle of the 20th century, vitamins have been produced as commodity chemicals and made widely available as inexpensive semisynthetic and synthetic-source multivitamin dietary supplements. Most vitamins are obtained with food, and a few vitamins, such as vitamin K, biotin, etc. are obtained by other means. For example, intestinal microorganisms (gut flora) produce vitamin K and biotin. Vitamin D is synthesized in the skin with the help of the natural ultraviolet wavelength of sunlight. Humans can produce some vitamins from dietary precursors. Examples include vitamin A, produced from beta carotene, and niacin, synthesized from the amino acid, tryptophan.

Deficiencies of vitamins are classified as either primary or secondary. When an organism does not get sufficient vitamins in food, a primary deficiency occurs. A secondary deficiency may be due to an underlying disorder that prevents or limits the absorption of the vitamin. It may also be due to a "lifestyle factor", such as excessive alcohol consumption and smoking.

Well-known human vitamin deficiencies include beriberi (thiamine deficiency), pellagra (niacin deficiency), scurvy (vitamin C deficiency), rickets (vitamin D deficiency), megaloblastic anemia (deficiency of folic acid), and pernicious anemia (deficiency of vitamin B_{12}). In large doses, some vitamins cause side effects such as nausea, diarrhea, and vomiting. The appropriate doses of vitamins differ by individual and are related to age and state of health.

VITAMIN A

Vitamin A is present only in foods of animal origin, and its provitamins (carotenes) are present in plants. The orange pigment of carrots, beta-carotene is used in the body to contribute to vitamin A levels. Alpha-carotene and gamma-carotene also have some vitamin activity. The compounds retinoids (retinol, retinal and retinoic acid) include the natural and synthetic forms of vitamin A. Retinol is vitamin A alcohol, retinal is vitamin A aldehyde, and retinoic acid is vitamin A acid.

Vitamin A: Absorption, Transport, and Metabolism

BAQ: Write a note on vitamin A: Absorption, transport, and metabolism.

Ans: Intestinal brush-border hydrolases and pancreatic hydrolases are responsible to hydrolyze dietary retinyl esters to release retinol and free fatty acids. Carotenes are hydrolyzed by the intestinal enzyme β-carotene 15–15′-dioxygenase to release two molecules of retinal, which are reduced to retinol.

Esterification of retinol takes place with free fatty acids in the intestinal mucosal cells. Esterified retinol gets incorporated into chylomicrons and enters the lymph, and is transported to the liver. It is stored in the liver and released as retinol according to the need of the body.

Zinc ions play an important role in the mobilization of retinol, which is circulated by binding with plasma retinol-binding protein (RBP) and in association with pre-albumin. The retinol–RBP complex binds to specific receptors on the peripheral tissue cell membrane and enters the target cell. A cellular retinol-binding protein carries retinol to the nucleus. Retinol binds to specific chromatin DNA and functions in manners similar to steroid hormones.

The RDA, Dietary Sources, Functions, Role

Competency achievement: The student should be able to:

PE12.1: Discuss the RDA, dietary sources, functions of vitamin A, and their role in health and disease

LAQ: Discuss the RDA, dietary sources of vitamin A, and their role in health and disease.

Ans: Following are the dietary sources of vitamin A:

Animal sources: Milk, egg yolk, liver, kidney, butter, cheese, fish (shark or cod liver oil), etc.

Vegetable sources (Provitamin A): Yellow and dark green vegetables and fruits, e.g.

papaya, carrots, spinach, mango, pumpkins, etc.

Recommended dietary allowance (RDA), per day:

Adults:

Male: 900 µg RAE

Women: 700 µg RAE

Pregnant women: 800 µg RAE

Lactating women: 1300 µg RAE

Children 1–3 years: 300 µg RAE

Children 4–9 years: 400 µg RAE

Children 10–15 years: 600 µg RAE

NOTE

1. **RAE** for vitamin A is given as retinol activity equivalents to account for the different bioactivities of retinol and provitamin A carotenoids.

   1 µg RAE is equivalent to 1 µg retinol, 2 µg supplemental beta-carotene, 12 µg dietary beta-carotene.
2. Vitamin A deficiency symptoms are not observed immediately since the hepatic stores can meet the body's requirement for 2–3 months.

Biochemical Functions of Vitamin A

1. Vitamin A plays a very important role in vision, proper growth, cell differentiation and turnover, reproduction, and maintenance of epithelial cells of the eye.

   The protein rhodopsin (Mol. wt. 35,000) is present in rods. It contains the protein opsin and 11-*cis* retinal.

   The initiation of the visual cycle depends on the ready availability of 11-*cis* retinaldehyde and hence vitamin A. In deficiency of vitamin A both the time taken to adapt to darkness and the ability to see in poor light are impaired.

   Rhodopsin is needed to see black and white as well as to see at night. Hence, a deficiency in vitamin A will inhibit the reformation of rhodopsin and lead to night blindness.
2. Maintenance of healthy epithelial tissue. Retinol and retinoic acid control keratin synthesis and maintain the moist surface of the skin by playing an important role in the synthesis of mucopolysaccharide of mucus secreted by epithelial cells.
3. Vitamin A plays an important role in the maintenance of the normal immune system.
4. Vitamin A is required for cholesterol synthesis, and the synthesis of transferrin and β-carotenes function as antioxidants.

Vitamin A Deficiency

A primary vitamin A deficiency is observed among children and adults who do not consume an adequate intake of food containing vitamin A.

Secondary vitamin A deficiency is associated with chronic malabsorption of lipids, impaired production and release of bile, low consumption of fat diets, and chronic exposure to oxidants (e.g. cigarette smoke).

Night blindness (nyctalopia) is the earliest symptom of vitamin A deficiency and severe deficiency leads to xerophthalmia. It is characterized by dryness in the cornea and conjunctiva and keratinization of epithelial cells. White triangular plaques (Bitot's spots) are seen in certain areas of the conjunctiva. Persistent xerophthalmia leads to corneal ulceration, degeneration, and death leading to blindness (keratomalacia).

Vitamin A deficiency also leads to impaired immunity, growth retardation, degeneration of germinal epithelium in males leading to sterility, decrease in plasma retinal bonding protein, keratinization of epithelial cells of the gastrointestinal tract, urinary tract, respiratory tract leading to increased bacterial infections and dryness of the skin.

Hypervitaminosis A

Excessive intake of vitamin A leads to its accumulation beyond the capacity of binding proteins. The unbound vitamin A causes tissue damage. The various symptoms of hypervitaminosis A include dermatitis, skeletal decalcification, liver enlargement, tenderness of long bones, irritability, weight loss, loss of hair and joint pain, etc.

Deficiency symptoms, diagnosis

SAQ: Enumerate the symptoms of vitamin A deficiency.

Ans: Inability to see in dim light, Bitot spots in eyes, dry skin, dry eyes, frequent infections, increased tendency to skin infections.

BAQ: Describe xerophthalmia under the following heads:

1. Diagnosis
2. Biochemical basis
3. Treatment

Ans: Xerophthalmia is a serious eye disorder. It is associated with vitamin A deficiency. It may lead to permanent blindness, if not treated in time.

1. Diagnosis: It is diagnosed by complete history related to poor vision by the patient in the darkness and eye examination, indicating dry eyes and the presence of bitot spots in the conjunctiva of the eyes. Bitot's spots are the presence of keratin deposits located superficially in the eyes (Fig 11.1). Low levels of serum retinol <20 µg/dl, indicate vitamin A deficiency.
2. Biochemical basis: Vitamin A deficiency, due to which vision and normal formation of epithelial tissue are affected.
3. Treatment: WHO, UNICEF, and Ministry of Women and Child Development. suggested prophylactic vitamin A dosage scheme on vitamin A supplementation is as follows:
   1. 100000 IU at 9 months with measles immunization.
   2. 200000 IU at 16–18 months with DPT booster.
   3. 200000 IU every six months up to the age of 5 years.

   1 IU = 0.3 µg of retinol.

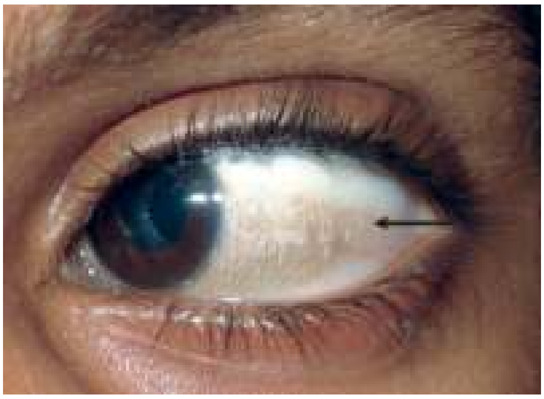


Fig. 11.1: Bitot's spots

SAQ: Write a brief note on skin disorders caused due to vitamin A deficiency.

Ans: A deficiency of vitamin A can cause dry, scaly, and itchy skin, due to affected normal formation of epithelial tissue. A deficiency of vitamin A may lead to the development of eczema, a clinical condition that causes dry, itchy, and inflamed skin. Dry skin is prone to bacterial and fungal infections.

Laboratory Method

BAQ: Give information on the laboratory determination of serum vitamin A and its normal range.

Ans: Serum vitamin A value does not decline until liver stores become critically depleted. Tests based on high-performance liquid chromatography (HPLC) are used to determine serum vitamin A.

By HPLC the reference interval (normal range) for serum beta-carotene is 10–85 µg/dl. Low values indicate vitamin A deficiency and elevated values are found in hypothyroidism (in which conversion of preformed vitamin A to vitamin A is decreased) and in diabetes mellitus due to associated hyperlipidemia.

BAQ: Describe events of "Wald's visual cycle".

Ans: The following are the events that represent "Wald's visual cycle".

1. Rhodopsin protein is present in the rod cells of the retina. It is a G-protein coupled receptor. Rhodopsin functions as the

primary photoreceptor molecule of vision, and contains an opsin molecule linked to a chromophore, 11-*cis*-retinal.

Vision begins with the photoisomerization of the 11-*cis*-retinal present in the rods. When the 11-*cis*-retinal chromophore absorbs a photon, it isomerizes from the 11-*cis* state to the all-*trans*-retinal. The absorbance spectrum of the chromophore depends on its interactions with the opsin protein to which it is bound.

2. Opsin undergoes a conformational change, which is responsible for the generation of nerve impulses, that reach the pretectum nucleus in the midbrain for the first visual relay in the brain.
3. All *trans*-retinal is immediately isomerized by the enzyme retinol dehydrogenase (of retinal epithelium) to 11-*cis*-retinal.
4. 11-*cis*-retinal combines with opsin and completes the visual cycle, with the termination of nerve impulses.
5. 11-*cis*-retinal is also converted to 11-*cis*-retinol in the liver.

BAQ: Describe the role of vitamin A in vision.

Ans: Vitamin A plays a very important role in vision, proper growth, cell differentiation, turnover, reproduction, and maintenance of epithelial cells of thse eye.

The retina of the human eye has the following two types of cells: (1) Rods (about 10 millions) and (2) cones (about 5 millions). The cones are at the center, and the rods are at the periphery of the retina. Cones are responsible for bright light and color vision, and rods are responsible for dim light vision. The protein rhodopsin (mol. wt. 35,000) is present in rods. It contains the protein opsin and 11-*cis* retinal.

Vision begins with the photoisomerization of the 11-*cis*-retinal present in the rods. When the 11-*cis*-retinal chromophore absorbs a photon, it isomerizes from the 11-*cis* state to the all-*trans*-retinal. The absorbance spectrum of the chromophore depends on its interactions with the opsin protein to which it is bound.

Opsin undergoes a conformational change, which is responsible for the generation of nerve impulses, that reach the pretectum nucleus in the midbrain for the first visual relay in the brain. The all-*trans*-retinal is immediately isomerized by the enzyme retinol dehydrogenase (of retinal epithelium) to 11-*cis*-retinal. 11-*cis*-retinal combines with opsin and completes the visual cycle, with the termination of nerve impulses.

The initiation of the visual cycle depends on the ready availability of 11-*cis*-retinaldehyde, which forms from vitamin A. In deficiency of vitamin A both the time taken to adapt to darkness and the ability to see in poor light are impaired.

Rhodopsin is needed to see black and white as well as to see at night. Hence, a deficiency in vitamin A will inhibit the reformation of rhodopsin and lead to night blindness.

THE NATIONAL PROPHYLAXIS PROGRAMME AGAINST NUTRITIONAL BLINDNESS

LAQ: Write a note on the National Prophylaxis Programme against Nutritional Blindness.

Ans: The National Prophylaxis Programme against Nutritional Blindness (NPPNB) due to vitamin A deficiency in India was started in 1970. The specific aim of NPPNB was to prevent nutritional blindness due to keratomalacia. This program was launched as an urgent remedial measure to control the high number of xerophthalmic blindness in India from the years 1950 to 1960. Clinical vitamin A deficiency declined drastically during the last 40 years, due to the NPPNB programs. There has been virtual disappearance of keratomalacia, and a sharp decline in the prevalence of Bitot spots.

In 1994, under the National Child Survival and Safe Motherhood (CSSM) Programme, the NPPNB due to vitamin A deficiency programme was modified because of the vulnerability of vitamin A deficiency in young children. The age group of eligible children for coverage was restricted to 9 to 36 months of age. Accordingly, each child was to receive five doses of vitamin A before 3rd birthday: For children aged 6–11 months, 1 dose of 100,000 IU of vitamin A, and for age 12–36 months of age one dose of 200,000 IU of vitamin A every six months. The administration of the first dose of vitamin A was linked to measles immunization.

In 2006, the age group of eligible children was revised as 6–59 months. This was done after reconsidering the recommendations of WHO, UNICEF, and the Ministry of Women and Child Development. The suggested prophylactic vitamin A dosage scheme was as follows:

1. 100000 IU at 9 months with measles immunization.
2. 200000 IU at 16–18 months with DPT booster.
3. 200000 IU every six months up to the age of 5 years.

1 IU = 0.3 µg of retinol.

NOTE

A national survey conducted by the Indian Council of Medical Research (ICMR) in 2001, covering 16 districts in all five regions of the country showed that only three out of 16 districts had a prevalence of Bitot spots (BS) of 0.5 percent and more.

Horizontal integration of vitamin A deficiency with anatomy, physiology and biochemistry

Anatomy

Eyes: Night blindness due to malformation of rhodopsin. Xerophthalmia due to dryness in the cornea and conjunctiva.

Skin: Malformation of epithelial tissue. Increase in the dryness of the skin. Keratinization of epithelial cells. Susceptibility to skin bacterial infections.

Respiratory tract: Effects on the respiratory tract due to increased keratinization of epithelial cells of the respiratory tract.

Male genital organs: Degeneration of germinal epithelium.

Kidneys: Change in glomerular filtration rate and reabsorption properties.

Lungs: Risk of pulmonary edema.

Physiology

- Affected Wald's visual cycle
- Affected normal functions of epithelial cells.

Biochemistry

Changed normal chemical nature of corneal epithelial tissue. Deficiency of vitamin A results in corneal epithelial tissue keratinization, ulceration, and necrosis. Vitamin A is necessary for the normal differentiation of non-squamous epithelial tissue and the prevention of excess keratinization of epithelial tissue. Abnormal keratinization is a consequence of vitamin A deficiency.

Vertical integration of vitamin A deficiency with para-clinical subjects

General medicine: Study of vitamin A deficiency pathophysiology.

Microbiology: Study of increased risk of microbial infections of skin and identification of related microorganisms.

Clinical pathology: Study of laboratory tests to find out the level of plasma vitamin A.

Preventive medicine: Study of prevention of vitamin A deficiency by balanced foods.

Vertical integration of vitamin A deficiency with clinical subjects

Pediatrics: Poor growth of the child. Examination of Bitot spots in the eyes.

Dermatology: Assessment of vitamin A deficiency to correlate with dry skin problems.

VITAMIN D

Vitamin D is a group of oil-soluble vitamins that resemble sterols in structure and function like a hormone. D_2 (ergocalciferol) and D_3

(cholecalciferol) are two major physiologically relevant forms of vitamin D, which are present in vegetables and non-vegetarian foods. Vitamin D_3 is also produced in the skin after the exposure of 7-dehydrocholesterol under the skin to ultraviolet B light from the sun or artificial sources.

Vitamin D Absorption, Transport and Storage

SAQ: Write a note on vitamin D absorption, transport and storage.

Ans: Vitamin D is an oil-soluble vitamin. Bile is required for the absorption of vitamin D in food, in the small intestine. Through lymph, it enters general blood circulation. Vitamin D binds to α-2 globulin and gets distributed throughout the body. A small amount of vitamin D is stored in the liver and other tissues.

BAQ: Write a note on the metabolism of vitamin D.

Ans: Cholecalciferol (D_3) and ergocalciferol (D_2) are present in the blood circulation following absorption of food lipids. 7-hydroxycholecalciferol present under the skin is also converted to cholecalciferol (D_3).

In the liver cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) are first hydrolyzed to 25-hydroxycholecalciferol (25-OH D_3) by a specific hydroxylase. 25-OH D_3 is the main circulatory and storage form of vitamin D. In the kidneys an enzyme 25-hydroxycholecalciferol 1-hydroxylase acts on 25-OH D_3 to produce 1, 25-dihydroxycholecalciferol (1, 25-DHCC, calcitriol).

Calcitriol is transported to various target organs by binding to a carrier protein, vitamin D-binding protein (VDBP). Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells. When calcitriol binds to VDR, it acts as a transcription factor that modulates the gene expression of transport proteins that are involved in calcium absorption in the intestine.

VDRs are expressed by cells in most organs, including the skin, heart, breast, brain, gonads, and prostate. VDR activation in the intestine, bone, kidney and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood with the influence of parathyroid hormone and calcitonin.

RDA, Dietary Sources of Vitamin D and Role

Competency achievement: The student should be able to:

PE12.6: Discuss the RDA, dietary sources of vitamin D, and their role in health and disease

LAQ: Discuss the RDA, dietary sources of vitamin D, functions and their role in health and disease.

Ans: Vitamin D: Dietary sources

Food sources such as fatty fish, eggs, and meat are rich in vitamin D and are often recommended for vitamin D. Milk is not a good source of vitamin D.

7-dehydrocholesterol is formed as an intermediate in cholesterol synthesis by the cells. It accumulates in the skin. On exposure to sunlight (UV light), 7-dehydrocholesterol is converted to pre-vitamin D. It is a non-enzymic reaction. Previtamin D undergoes a further reaction to form cholecalciferol.

Recommended dietary allowance (RDA)

Adults: The daily requirement of vitamin D is 400 IU or 10 µg per day of cholecalciferol. In countries with good sunlight (such as India) vitamin D RDA is 200 IU (5 µg of cholecalciferol).

Children: 600 IU per day

Functions of Vitamin D

1. The main function of vitamin D is the control of calcium homeostasis. Similarly, the concentration and metabolism of vitamin D are regulated by factors that

respond to plasma concentrations of calcium and phosphorus. The principal function of vitamin D is to maintain the plasma calcium concentration and normal bone formation in the following ways:

Vitamin D

A. Increases intestinal absorption of calcium and phosphorus.

B. Decreases urinary excretion of calcium by stimulating resorption in the distal renal tubules.

C. Plays an important role in normal bone formation. In the osteoblasts of bone, calcitriol stimulates calcium uptake. Calcium phosphate is deposited in the formation of bone. It promotes the healthy mineralization, growth, and remodeling of bone.

2. *Role of vitamin D in strengthening the immune system:* The most important components of the innate immune system, i.e. macrophages, T-lymphocytes, dendritic cells, and natural killer cells express vitamin D receptors (VDRs). Calcitriol binds to these receptors and expresses autocrine, paracrine, and endocrine properties. Immune cells secrete anti-inflammatory interleukins (IL-4, IL-6, IL-10, etc.), which bind proinflammatory interleukin receptors (IL-1) and reduce infection-induced inflammation.

   Similarly, by the effects of calcitriol, TH2 helper cells become more active, which promotes the activation of B cells to secrete antibodies and decreases the activities of TH1 helper cells, which promote inflammatory reactions in infections.

   By the action of calcitriol, cytotoxic lymphocytes secrete specific proteins, perforins, and granzymes, which are responsible for destroying viruses and other microorganisms by creating holes in their specific structures. Many infected host cells undergo apoptosis, and viral spread decreases.

3. Other functions of vitamin D in the form of calcitriol are as follows: Regulation of insulin secretion, synthesis and secretion of thyroid and parathyroid hormones, modulation of cell proliferation, modulation of neuromuscular function, and influence on the action of many genes that regulate the proliferation, differentiation, and apoptosis of cells.

Deficiency, Symptoms and Diagnosis

Deficiency of Vitamin D: A deficiency of vitamin D leads to rickets in children and osteomalacia in adults. Rickets and osteomalacia are characterized by impeded growth, and deformity, of the long bones. Bone deformities are caused mainly by incomplete mineralization. This results in soft and pliable bones.

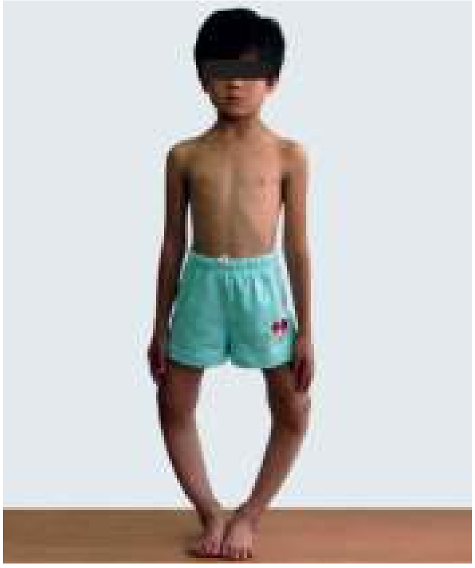


Fig. 11.2: Bone deformities in rickets

NOTE

Refer to **Ch 8** for detailed information on rickets.

Hypervitaminosis D

Overdoses of vitamin D lead to toxic effects on the body. It leads to increased calcium in the blood circulation (hypercalcemia). Due to prolonged hypercalcemia, calcium deposits in the soft tissues, arteries, and kidneys (in the form of stones). Hypervitaminosis D leads to weight loss, nausea, loss of appetite, and increased thirst.

Laboratory Method

BAQ: Give information on laboratory tests of serum vitamin D and its reference range.

Ans: The serum concentration of 25-hydroxy-vitamin D is used to determine vitamin D status. It reflects vitamin D produced in the skin as well as that acquired from the diet. 25-hydroxy-vitamin D has a fairly long circulating half-life of 15 days. It does not, however, reveal the amount of vitamin D stored in other body tissues.

The reference interval (normal range) of serum 25-hydroxy-vitamin D is 10–65 ng/ml. A level of serum 25-hydroxy-vitamin D lower than 10 ng/ml (25 nmol/L) is associated with the most severe deficiency diseases, such as rickets in infants and children, and osteomalacia in adults.

A concentration above 15 ng/ml (37.5 nmol/L) is generally considered adequate for those in good health. Levels of 25-hydroxy-vitamin D that are consistently above 200 ng/mL (500 nmol/L) are thought to be potentially toxic.

Most assays of serum 25-hydroxy-vitamin D (and also 1,25-dihydroxy-vitamin D) involve deproteinization of serum, purification of separated vitamin D components, and determination by using UV absorption methods, RIA, or EIA methods.

Horizontal integration vitamin D deficiency with pre-medical subjects

Anatomy: Skeletal system: Poor formation of bones due to calcium and phosphorus deficiency.

Nervous system: In the nervous system, vitamin D deficiency is involved in poor calcium trafficking, defective synthesis of synaptic structural proteins, neurotrophic factors, and deficiency in the synthesis of neurotransmitters.

Musculoskeletal system: Deficiency of vitamin D leads to secondary hyperparathyroidism, increases bone loss, and calcium loss that leads to a weak musculoskeletal system.

Physiology

- Affected absorption of calcium and inorganic phosphorus in the intestine and kidneys.
- Parathyroid gland hyperactivity.
- Poor circulation of calcium and inorganic phosphorus in the body.
- Poor functioning of immune cells.

Biochemistry

Disturbed metabolism of calcium and inorganic phosphorus

Vertical integration of vitamin D deficiency with para-clinical subjects

General medicine*:* Study of rickets and Osteomalacia pathophysiology.

Microbiology: Study of increased risk of microbial infections due to immunocompromised conditions caused by vitamin D deficiency. For example, COVID-19, diarrhea caused by microbial infections.

Clinical pathology*:* Laboratory tests to find out the level of plasma vitamin 25-hydroxycholecalciferol, serum calcium, and inorganic phosphorus.

Preventive medicine*:* Study of prevention of rickets and osteomalacia by vitamin D drugs and food supplements.

Vertical integration of vitamin D deficiency with clinical subjects

Pediatrics: Consideration of poor growth of the child and malformation of bones.

Obstetrics and gynecology*:* Support of sufficient food supplements containing vitamin D and drugs during pregnancy and for the normal growth of fetus and baby.

Dermatology: Assessment of vitamin D deficiency to correlate with increased skin infections.

VITAMIN E

Vitamin E is a family of naturally occurring oil-soluble vitamins, that exists in eight chemical forms: Four tocopherols such as α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, etc. and four tocotrienols such as α-tocotrienols, β-tocotrienols, γ-tocotrienols and δ-tocotrienol. α-tocopherol is the most active form of vitamin E as it has the highest bioavailability.

Absorption and Metabolism

SAQ: Write a brief note on the absorption and metabolism of vitamin E.

Ans: Vitamin E in food is absorbed during lipid digestion which requires bile. Through lymph, it enters general blood circulation and is taken to the liver. In the liver, vitamin E is incorporated in VLDL and LDL and transported to various tissues. It is stored in the liver, adipose tissue and muscles.

RDA, Dietary Sources of Vitamin E and Role

Competency achievement: The student should be able to:

PE12.11: Discuss the RDA, dietary sources of vitamin E, and their role in health and disease

LAQ: Discuss the RDA, functions, deficiency symptoms, dietary sources of vitamin E, and their role in health and disease.

Ans: Dietary resources: It is present in milk, butter, eggs, meat, almonds, nuts, green leafy vegetables, papayas, sweet potatoes, broccoli, and mangoes. Various vegetable oils, such as sunflower oil.

Recommended dietary allowance (RDA), per day

Daily consumption of vitamin E (mainly through polyunsaturated fatty acids) of about 15 IU (10 mg) for men and 12 IU (8 mg) for women is recommended.

Functions of Vitamin E

The most important functions of vitamin E are attributed to its antioxidant property. The following are various functions of vitamin E:

1. Vitamin E (mainly α-tocopherol) protects cell membranes from oxidation by reacting with oxidative radicals produced in the lipid peroxidation chain reactions. It removes the free radical intermediates and prevents the initiation of oxidation reactions. The oxidized α-tocopherol radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate and retinol.
2. It prevents the peroxidation of unsaturated fatty acids.
3. It protects the cell membrane of red blood cells from the damaging effects of oxidants.
4. It prevents oxidation of vitamin A, carotenes, and LDL.
5. It preserves and maintains the germinal epithelium of gonads for proper reproductive functions. It plays an important role in the prevention of sterility.
6. It plays an important role in the synthesis of heme by increasing the activity of δ-aminolevulinic acid (ALA) synthase and ALA dehydratase.
7. It is required for normal absorption of amino acids in the intestine.
8. It protects the liver from the effects of toxic substances.

Deficiency Symptoms and Diagnosis

Vitamin E deficiency symptoms: The following are some of the vitamin E deficiency symptoms: Degenerative changes in muscles, muscular weakness, impaired reflexes, and coordination, changes in the central nervous system, and decreased lifespan of red blood cells, leading to anemia, etc. These symptoms are mainly related to a defect in fat absorption and transport.

NOTE

The toxic effects of vitamin E are rarely seen.

Laboratory Method

SAQ: Give a normal range of serum vitamin E and describe the laboratory method for its determination.

Ans: Determination of serum tocopherols is done by high-performance liquid chromatography (HPLC) method.

In the case of adults the reference interval (normal range) of serum vitamin E is 0.5 to 1.8 mg/dl, and in the case of children (1–16 years), it is 0.6–1.0 mg/dl.

VITAMIN K

Vitamin K is a group of oil-soluble vitamins. The main type is K_1 (phylloquinone), found in green leafy vegetables such as kale and spinach. The other type is K_2 (menaquinones), which is present in cheese, meat, and eggs and is also produced by bacterial flora in the small intestine. There are three synthetic forms of vitamin K, vitamins K_3, K_4, and K_5, from which some forms are used in the pet food industry (vitamin K_3), and to inhibit fungal growth (vitamin K_5).

Absorption and Metabolism

SAQ: Write a brief note on the absorption and metabolism of vitamin K.

Ans: Vitamin K in the diet is absorbed during lipid digestion which requires the presence of bile. It (K_2) is also synthesized by intestinal bacteria. Through lymph, it enters general blood circulation and is taken to the liver. In the liver, vitamin K is incorporated in LDL and transported to various tissues. It is stored mainly in the liver and adipose tissue.

Dietary sources of vitamin K and their role

Competency achievement: The student should be able to:

PE12.14: Discuss the RDA, dietary sources of vitamin K, and their role in health and disease

LAQ: Discuss the RDA, dietary sources of vitamin K, and their role in health and disease.

Ans: Dietary sources: Vitamin K is present in egg yolk, meat, liver, cheese, tomatoes, cabbage, alpha alpha, spinach, and other green vegetables.

Recommended Dietary Allowance (RDA)

For adults, RDA is 70–140 µg per day. Since it is synthesized in the gut, it is recommended that at least half the requirement of vitamin K should be provided through food.

Functions of vitamin K: Vitamin K acts as a specific coenzyme for the post-translational modification of certain proteins, mostly required for blood coagulation but also involved in metabolic pathways in bone and other tissue.

Vitamin K is involved in the carboxylation of certain glutamate residues in proteins to form gamma-carboxyglutamate residues (Gla-residues). Negatively charged Gla-residues formed by the action of vitamin K combine with positively charged calcium ions to form a complex, which plays an important role in the blood coagulation mechanism. For example, in the case of prothrombin, when it combines with Gla-residue and calcium complex and gets attached to the phospholipids on the surface of platelets. This leads to the conversion of prothrombin to thrombin, and then thrombin initiates the conversion of fibrinogen (soluble form) to fibrin (insoluble form).

Vitamin K Deficiency Symptoms, Diagnosis

Primary vitamin K deficiency is rare in healthy adults. However, newborn infants are at an increased risk of deficiency. Other populations with an increased prevalence of secondary vitamin K deficiency include patients with cystic fibrosis, inflammatory bowel diseases, patients who had abdominal surgeries, persons suffering from liver disease (e.g. alcoholics), and patients under anticoagulant therapy.

Blood coagulation is adversely affected by the deficiency of vitamin K. An individual with vitamin K deficiency may bleed profusely, even with minor injuries. Blood bleeding time, clotting time, and prothrombin time may increase vitamin K deficiency.

Toxic effects of hypervitaminosis K: There is no known toxicity associated with high doses of the phylloquinone (vitamin K_1) or menaquinone (vitamin K_2) forms of vitamin K, and therefore, no tolerable upper intake level (UL) has been set. However, a synthetic form of vitamin K, vitamin K_3 (menadione), is toxic. Large doses of K_3 have been shown to cause, hemolytic anemia, allergic reactions, and cytotoxicity in liver cells.

SAQ: Enumerate laboratory tests that indicate vitamin K deficiency.

Ans: Increase in blood bleeding time, clotting time, and increase in prothrombin time indicate vitamin K deficiency.

SAQ: Explain the anticoagulant action of the drug warfarin and its safe use.

Ans: Warfarin (coumadin) is an anticoagulant. Warfarin decreases blood coagulation by inhibiting vitamin K epoxide reductase. This enzyme recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. For this reason, drugs in this class are also referred to as vitamin K antagonists. A few years after its introduction, warfarin was found to be effective and relatively safe for preventing thrombosis and embolism (abnormal formation and migration of blood clots) in many disorders. It was approved for use as a medication in the early years of 1950. Since it is difficult to predict the appropriate dose of warfarin which gives the desired degree of suppression of the clotting, warfarin treatment must be carefully monitored to avoid overdosing.

Laboratory Method

SAQ: Describe the method used for the determination of serum vitamin K and its reference range.

Ans: Reference range: The reference interval (normal range) for serum vitamin K is 0.13–1.19 ng/ml.

Determination of serum vitamin K is performed by serum protein precipitation, followed by lipid extraction and HPLC with electrochemical or fluorometric detection.

WATER-SOLUBLE VITAMINS

BAQ: Write a note on water-soluble vitamins.

Ans: B-complex vitamins and vitamin C are water-soluble vitamins that are not stored in the body and must be replaced each day. These vitamins are easily destroyed during food storage and preparation. The B-complex group is found in a variety of foods such as meat, eggs, fish, poultry, fish, cereal grains, milk, legumes, and fresh vegetables. Citrus fruits are good sources of vitamin C. Proper storage and preparation of food can minimize vitamin loss. To reduce water-soluble vitamin loss, it is necessary to refrigerate the food products. Milk and grains should be stored away from strong light, and the cooking water from vegetables should be used to prepare soups.

The following eight water-soluble vitamins are known as the B-complex vitamins: Thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6, biotin, folic acid, and vitamin B_{12}. These vitamins function as coenzymes and help the body to obtain energy from food. They are also important for normal healthy skin, appetite, good vision, a healthy nervous system, and normal red blood cell formation. Beriberi, pellagra, and pernicious anemia are three important B-vitamin deficiencies. Among the nutrients added during the enrichment process are niacin, riboflavin, thiamin, and folate (with iron). Some examples of enriched grain products are white rice, many breakfast cereals, white flour, breads, and pasta.

VITAMIN C

Vitamin C (L-ascorbic acid or L-ascorbate) is a water-soluble vitamin. It is a hexose derivative and a strong reducing agent. L-ascorbic acid undergoes oxidation to form dehydroascorbic acid. This reaction is reversible, and both these forms are biologically active.

Vitamin C is an essential vitamin for humans. Many animals can synthesize vitamin C from glucose. However, humans, other primates, guinea pigs, and bats cannot synthesize vitamin C. In living organisms, vitamin C is an antioxidant, since it protects the body against oxidative stress. It is also a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions. When L-ascorbate, carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate, which can be reduced back to the active L-ascorbate form in the body by enzymes and glutathione.

Absorption and Metabolism of Vitamin C

BAQ: Write a note on the absorption and metabolism of vitamin C.

Ans: Vitamin C present in food is rapidly absorbed from the intestine, and it enters general blood circulation. It is absorbed in the body by both active transport and simple diffusion. Two sodium-dependent active transports, sodium-ascorbate co-transporters (SVCTs) and hexose transporters (GLUTs) are required for the absorption of vitamin C. Vitamin C is not stored in the body to any significant extent. The concentration of vitamin C in the plasma larger than the renal threshold is rapidly excreted in the urine with a half-life of about 30 minutes. Excess vitamin C can be oxidized and broken down in the human body by the enzyme L-ascorbate oxidase.

RDA, Dietary Sources, Functions and Role

Competency achievement: The student should be able to:

PE12.19: Discuss the RDA, dietary sources, functions of vitamin C, and their role in health and disease

LAQ: Discuss the RDA, dietary sources of vitamin C, functions, and their role in health and disease.

Ans: Dietary sources: Tomatoes, potatoes, cabbage, spinach, citrus fruits (oranges, lemon, etc.), gooseberry (amla), papaya, kiwifruit, guava, etc.

Recommended Dietary Allowance (RDA): Children age 4 to 8 years: Average 25 mg/day

Boys and girls ages 9 to 13 years: Average 45 mg/day

Adults: 60–70 mg/day

The following are various functions of vitamin C:

1. It plays an important role as a coenzyme in the synthesis of collagen in the following enzymatic reactions: Hydroxylation of proline catalyzed by prolyl hydroxylase and hydroxylation of lysine catalyzed by lysyl hydroxylase. Both these reactions require vitamin C as a coenzyme. In the process, proto-collagen (precursor protein of collagen) is converted to collagen.
2. Vitamin C is required for bone formation, which involves collagen, organic matrix, calcium, phosphates, etc.
3. Vitamin C increases iron absorption in the small intestine by keeping it in the ferrous form.
4. Vitamin C is required for the formation of tetrahydrofolate, which is required for the maturation of red blood cells.
5. It plays an important role in the synthesis of serotonin from tryptophan, and the synthesis of carnitine from L-lysine. Carnitine is essential for the transport of fatty acids into mitochondria for ATP generation.

Deficiency Symptoms, Diagnosis

Deficiency of vitamin C: A deficiency of vitamin C leads to the disease scurvy (Fig. 11.3). The following are the specific symptoms of scurvy: Spongy and purplish gums that are prone to bleeding, loose teeth, scaly, dry and brownish skin, very dry hair, bulging eyes (proptosis) and severe and easy bruising.

BAQ: Describe scurvy under the following heads:

1. Symptoms
2. Diagnosis
3. Biochemical basis
4. Treatment and
5. Prevention of vitamin C deficiency.

Ans:

1. Symptoms: Scurvy is a disease caused due to vitamin C (ascorbic acid) deficiency. Following are specific symptoms of scurvy: Spongy and purplish gums that are prone to bleeding, loose teeth, scaly, dry, and brownish skin, very dry hair, bulging eyes (proptosis), and severe and easy bruising.

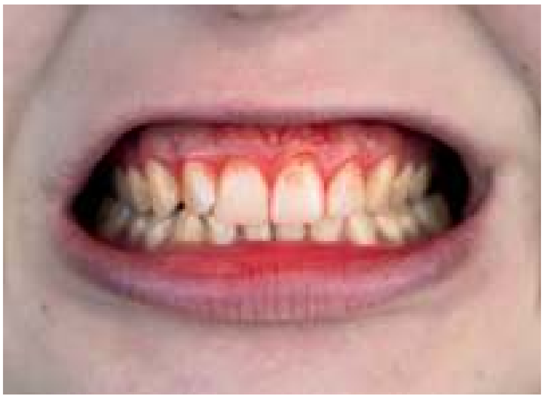


Fig. 11.3: Bleeding gums in scurvy

2. Diagnosis: It is based on physical signs: Examination of gums: Spongy and purplish and bleeding gums. Dermatologic findings include poor wound healing, mucocutaneous petechiae, and ecchymosis. X-ray examinations, and improvement in scurvy-related clinical conditions after treatment.
3. Biochemical basis: Vitamin C plays an important role as a coenzyme in the synthesis of collagen. Normal collagen is a primary structural protein in the human body, necessary for healthy blood vessels, muscle, skin, bone, cartilage, and other connective tissues. Defective connective tissue due to vitamin C deficiency leads to fragile capillaries, resulting in abnormal bleeding, bruising, and internal hemorrhage. Collagen is an important part of a bone, hence bone formation is also affected. Defective collagen fibrillogenesis impairs wound healing.
4. Treatment is with vitamin C supplements taken by mouth. Recommended average doses of vitamin C are 100 mg per day. Improvement often begins in a few days with complete recovery in a few weeks. Sources of vitamin C in the diet include citrus fruit and a number of vegetables such as tomatoes and potatoes. It is important to note that cooking often decreases vitamin C in foods.
5. Prevention of vitamin C deficiency: Intake of foods rich in vitamin C.

BAQ: Enumerate various roles of vitamin C in metabolic reactions.

Ans:

1. Vitamin C is needed for a variety of biosynthetic pathways such as hydroxylation and amidation reactions.
2. Vitamin C is required for the formation of tetrahydrofolate, which is required for the maturation of red blood cells.
3. Vitamin C plays an important role in the synthesis of serotonin from tryptophan.
4. Vitamin C plays an important role in the synthesis of carnitine from L-lysine. Carnitine is essential for the transport of fatty acids into mitochondria for ATP generation.

Laboratory Method

SAQ: What is the reference range and test used to determine serum vitamin C?

Ans: Reference range: 0.6–2 mg/dl

Laboratory determination of serum vitamin C: Colorimetric method.

Oxidation of serum vitamin C with 2,4 dinitrophenyl hydrazine forms red bis-hydrazone, which can be measured photometrically, using a known standard.

Horizontal integration of vitamin C deficiency with pre-clinical subjects

Anatomy: Adverse effects of deficiency of vitamin C on the formation of bones, maturation of red blood cells, and formation of connective tissues.

Physiology: Affected functions of the skin, blood vessels, red blood cells, and bone cells.

Biochemistry: Hydroxylation of proline catalyzed by prolyl hydroxylase and hydroxylation of lysine catalyzed by lysyl hydroxylase is affected since both these reactions require vitamin C as a coenzyme.

Vertical integration of vitamin C deficiency with para-clinical subjects

General medicine: Study of effects of vitamin C deficiency-related pathophysiology.

Preventive medicine: Prescriptions of food and supplements to prevent vitamin C deficiency.

Horizontal integration of vitamin C deficiency with clinical subjects

Pediatrics: Correlation of teeth and gum bleeding episodes in children with vitamin C deficiency.

B COMPLEX VITAMINS

The B complex vitamins are a group of water-soluble vitamins that play important roles in cell metabolism. B vitamins in the past were thought to be a single vitamin, referred to as vitamin B. Later on, research on these vitamins showed that they are chemically distinct vitamins that often coexist in the same foods. In general, supplements containing all eight are referred to as a vitamin B complex. Following all B vitamins are water-soluble, and are dispersed throughout the body. Most of the B vitamins must be replenished regularly since any excess is excreted in the urine.

THIAMINE

All living organisms require thiamine as an important component of food. However, it is synthesized in bacteria, fungi, and plants. Insufficient intake results in a disease called Beriberi that affect the peripheral nervous system and the cardiovascular system.

Absorption and Metabolism

BAQ: Write a note on the absorption and metabolism of thiamine.

Ans: Absorption and metabolism of thiamine in food take place as follows:

1. Thiamine present in the food is released by the action of phosphatase and pyrophosphatase in the upper small intestine. At low thiamine concentrations, the process is carrier-mediated. However, at higher concentrations, absorption occurs via passive diffusion. Active transport is greatest in the jejunum and ileum. Active transport is inhibited by alcohol consumption. A decrease in thiamine absorption occurs at a higher intake above 5 mg.
2. After absorption in general blood circulation, the majority of thiamine binds to serum proteins, mainly albumin. Approximately 90% of the total thiamine in the blood is in erythrocytes.
3. Uptake of thiamine by cells of the blood and other tissues occurs by active transport and passive diffusion. About 80% of intracellular thiamine is phosphorylated and most are bound to proteins. Storage of thiamine is about 25 to 30 mg with the greatest concentrations in the liver, kidneys, skeletal muscle, and brain. Thiamine and its acid metabolites are excreted mainly in the urine.

RDA, Dietary Sources of Thiamine and Role

Competency achievement: The student should be able to:

PE12.15: Discuss the RDA, dietary sources, functions of vitamins of B-complex, and their role in health and disease

LAQ: Discuss the RDA, dietary sources, functions of vitamins of thiamine, and its role in health and disease.

Ans: Dietary sources: Nuts, cereals, pulses, oils seed, yeast, milk, pork, liver, heart, kidney, etc. It is concentrated in the outer layer of cereals.

Recommended Dietary Allowance (RDA)

- Adults: 1–1.5 mg per day
- Children: 0.7–1.2 mg per day
- In the case of pregnant and lactating women and also in the case of alcoholics and old persons RDA is 2 mg per day.

Functions and deficiency symptoms

Thiamine derivatives and thiamine-dependent enzymes are present in all cells of the body. Hence, a thiamine deficiency adversely affects all of the organ systems. However, the nervous system and the heart are particularly sensitive to thiamine deficiency, due to their high oxidative metabolism.

Thiamine deficiency, diagnosis

Deficiency of thiamine can be caused by malnutrition, a diet high in thiaminase-rich foods (raw freshwater fish, raw shellfish, etc.), and foods high in anti-thiamine factors (tea, coffee, betel nuts).

Thiamine deficiency may also be caused in case of seriously impaired nutritional status associated with chronic diseases, such as AIDS, alcoholism, gastrointestinal diseases, and persistent diarrhea and vomiting

Beriberi

Thiamine deficiency leads to beriberi, a neurological and cardiovascular disease. The early symptom of thiamine deficiency is weakness, anorexia, nausea, irritability, mental depression and peripheral neuropathy. The three major forms of beriberi are:

1. Dry beriberi,
2. Wet beriberi, and
3. Infantile beriberi.

1. **Dry beriberi** is characterized mainly by peripheral neuropathy. In this clinical condition, symmetric impairment of sensory, motor, and reflex functions is observed, which affects distal (more than proximal) limb segments and causes calf muscle tenderness.
2. **Wet beriberi** is associated with mental confusion, edema, tachycardia, muscular wasting, cardiomegaly, and congestive heart failure in addition to peripheral neuropathy.
3. **Infantile beriberi** occurs in infants breast-fed by mothers who are thiamine-deficient (who may not show symptoms of thiamine deficiency). Infants may manifest cardiac and aphonic forms of the disorder. Infants with cardiac beriberi frequently exhibit a loud piercing cry, tachycardia, and vomiting. Convulsions are not uncommon, and death may take place if appropriate thiamine therapy is not given.

Wernicke's encephalopathy

Wernicke's encephalopathy is similar to dry beriberi, caused due to thiamine deficiency. It may also occur in patients with impaired nutrition from other causes, such as in the case of patients with AIDS and severe gastrointestinal disease. Wernicke's encephalopathy is a neuro-psychiatric disorder characterized by paralysis of eye movements, abnormal stance and gait, and markedly deranged mental function.

Treatment

Intravenous (IV) or orally (PO): 50 mg thiamine three times daily until symptoms resolve. Later on 10 mg/day oral tablets of thiamine with additional food supplements, until the expected recovery is complete.

Following thiamine treatment, rapid improvement in the clinical conditions occurs in one or two days. However, improvements in peripheral neuropathy require several months of thiamine treatment.

BAQ: Enumerate the various biochemical changes that occur in thiamine deficiency:

Ans: The following are the various biochemical changes that occur in thiamine deficiency:

1. Pyruvic acid, which forms from glucose, requires thiamine as a coenzyme for

further conversion into acetyl-CoA for the completion of the citric acid cycle. In the deficiency of thiamine, pyruvic acid accumulates in the tissues and may cross the blood–brain barrier leading to polyneuritis.

2. Since the citric acid cycle is affected, due to the depleted level of ATP, the cellular functions are adversely affected.
3. Transketolase of pentose phosphate pathways (which yield NADPH) also requires thiamine as a cofactor. Depletion of NADPH also affects cellular activities mainly related to fatty acid synthesis.
4. Impairment in nerve impulse transmission takes place in thiamine deficiency.

BAQ: Enumerate reasons that lead to thiamine deficiency in alcoholics.

Ans: Alcoholics may have thiamine deficiency because of the following reasons:

1. Inadequate nutritional intake
2. Decreased uptake of thiamine from the GI tract due to alcohol influence.
3. Liver thiamine stores are reduced due to hepatic steatosis or fibrosis.
4. Impaired thiamine utilization: For the activation of thiamine-dependent enzymes, magnesium ions are required, which are deficient in chronic alcoholics.
5. Ethanol inhibits thiamine transport in the gastrointestinal system and blocks phosphorylation of thiamine to its cofactor form (ThDP).

SAQ: Give two reasons that lead to the loss of thiamine from foods.

Ans:

1. Polishing of rice removes about 80% of thiamine. During cooking, it is extracted in water.
2. Sulfites added to foods as a preservative destroys thiamine. It attacks thiamine at the methylene bridge in the structure, cleaving the pyrimidine ring from the thiazole ring.

Laboratory Determination

BAQ: Give information on the reference range of thiamine in blood and blood test.

Ans:

The reference interval (normal range) of TPP in whole blood is 90–140 nmol/L (or 275–675 ng/g Hb).

The reference range of erythrocyte TPP is 173 to 293 nmol/liter erythrocytes (or 280–590 ng/g Hb).

Plasma (or serum) concentration reflects recent intake of thiamine and mainly in the unphosphorylated form at the concentration of 10–20 nmol/L.

Erythrocytes contain about 80% of the total thiamine of whole blood.

Laboratory test: Determination of blood TPP is performed by a protein precipitation step, followed by the formation of a fluorophore in an alkaline condition with potassium ferricyanide and fluorometric measurement. For erythrocyte TPP determination, washed cells are used.

Horizontal integration of thiamine deficiency with pre-clinical subjects

Anatomy: Peripheral neuropathy

Muscular system: Muscle wasting

Heart: Cardiomegaly

Red blood cells: Normal growth

Gastrointestinal tract: Gastrointestinal disturbances

Physiology: Pyruvic acid accumulation in the tissues. Affected cellular activities due to depletion of NADPH. Impairment in nerve impulses.

Biochemistry: Due to depleted levels of ATP the cellular functions are adversely affected. Fatty acid synthesis is affected due to the depletion of NADPH.

Vertical integration of thiamine deficiency with para-clinical subjects

General medicine: Study of thymine deficiency

Preventive medicine*:* Prescriptions of food and supplements to prevent thymine deficiency.

Vertical integration of thiamine deficiency with clinical subjects

Gynecology and obstetrics*:* General health of a woman and additional intake of thiamine required during pregnancy.

Pediatrics: Correlation of neurological disorders in children with thymine deficiency.

RIBOFLAVIN

Riboflavin is the central component of the coenzymes flavin adenine mononucleotide (FMN) and flavin adenine dinucleotide (FAD) required for various enzyme actions. Riboflavin is required for a wide variety of cellular processes. It plays an important role in energy metabolism and the metabolism of proteins, fats, ketone bodies, and carbohydrates.

Riboflavin imparts the yellow-orange color to B-vitamin preparations. The urine of a person who is treated with vitamin B-complex tablets, drinks, or solutions appears fluorescent yellow due to the excretion of excess riboflavin in urine.

Absorption and Metabolism

SAQ: Write a note on the absorption and metabolism of riboflavin.

Ans: Riboflavin present in food is absorbed in the small intestine and enters general circulation, and is taken up by the cells for the synthesis of cofactors FMN and FAD, which function as cofactors for a wide variety of oxidative reactions. Flavins can act as oxidizing agents because of their ability to accept a pair of hydrogen atoms. Reduction of FAD and FMN yields the reduced forms FMNH2 and FADH2. Enzymes that use FMN or FAD are called flavoproteins, which exhibit a wide range of redox potential.

RDA, Dietary Sources, Functions, Role

Q: Discuss the RDA, dietary sources of riboflavin, and its role in health and disease.

Ans: Dietary sources: Liver, milk, cheese, almonds, leafy green vegetables, kidneys, yeast, legumes, tomatoes and mushrooms, are good sources of vitamin B_2.

Recommended Daily Allowance (RDA)

For adults recommended daily allowance of riboflavin is 1.2–1.7 mg. For pregnant and lactating females, 1.5–2.0 mg riboflavin is recommended.

Functions of riboflavin in the form of coenzyme and flavoprotein:

1. FAD is required to convert retinal (vitamin A) to retinoic acid.
2. Flavoproteins play very important roles in the electron transport chain.
3. FAD is required to convert tryptophan to niacin (vitamin B_3).
4. Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) requires FAD, which is important to maintain the integrity of the red blood cell membrane.
5. Decarboxylation of pyruvate and α-ketoglutarate requires FAD.
6. FAD is required for the synthesis of pyridoxic acid from pyridoxal (vitamin B_6).
7. Synthesis of an active form of folate (5-methyl THF) is dependent on FADH2.
8. Fatty acyl-CoA dehydrogenase requires FAD in fatty acid oxidation.

Deficiency, Symptoms, Diagnosis

Riboflavin deficiency and symptoms: A deficiency of riboflavin can be primarily due to inadequate vitamin intake. A secondary riboflavin deficiency may occur due to certain clinical conditions such as prolonged diarrhea, that affect the absorption of riboflavin in the intestine.

Signs and symptoms of riboflavin deficiency include cheilosis, glossitis, and dermatitis (Fig. 11.4). The symptoms observed in cheilosis are cracked red lips, inflammation of the lining of the mouth, and cracks at the

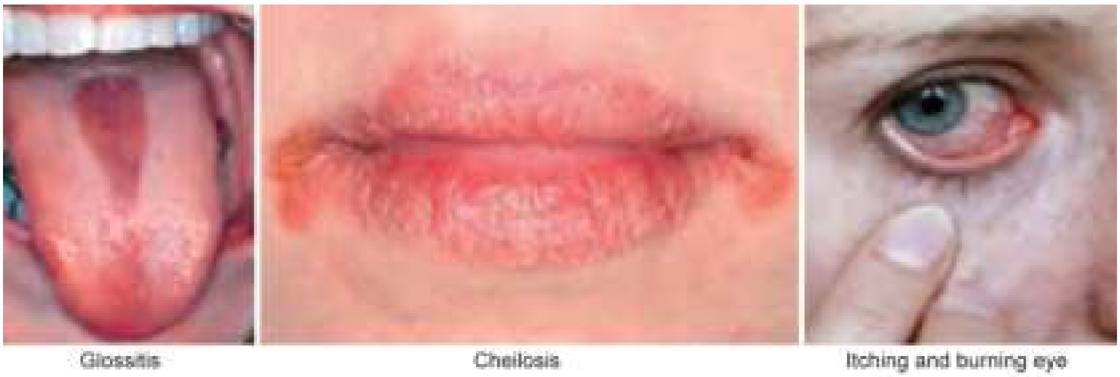


Fig. 11.4: Riboflavin deficiency symptoms

corners of the mouth (angular cheilitis). In glossitis, inflammation of the tongue is observed with purplish color. In riboflavin deficiency dermatitis, dry and scaling skin is observed with fluid in the mucous membranes. The eyes may also become itchy, watery, and sensitive to bright light.

Laboratory Test

BAQ: Give information on the normal range of serum riboflavin and laboratory test.

Ans: The reference interval (normal range) of serum (or plasma) riboflavin is 4–24 µg/dl. The reference interval of erythrocyte riboflavin is 10–50 µg/dl.

Laboratory Determination of Riboflavin

After protein precipitation, the HPLC technique is used for the separation of coenzyme form (FMN, FAD), followed by measurement of the intensity of fluorescence by a fluorometer.

BAQ: Enumerate four important functions of flavoproteins.

Ans:

1. FAD is required to convert retinal (vitamin A) to retinoic acid.
2. FAD is required to convert tryptophan to niacin (vitamin B_3).
3. Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) requires FAD, which is important to maintain the integrity of the red blood cell membrane.
4. FAD is required for the synthesis of pyridoxic acid from pyridoxal (vitamin B_6).

Horizontal integration of thiamine deficiency with pre-clinical subjects

Anatomy

Mouth: Affected anatomy. Cheilosis: Cracked red lips, inflammation of the lining of the mouth, and cracks at the corners of the mouth (angular cheilitis). Glossitis, inflammation of the tongue.

Peripheral nervous system: Neuropathy.

Skin: Dermatitis, dry and scaling skin with fluid in the mucous membranes.

Eyes may also become itchy, watery, and sensitive to bright light.

Muscular system: Muscle wasting

Heart: Cardiomegaly

Red blood cells: Affected normal growth

Gastrointestinal tract: Gastrointestinal disturbances

Physiology: Affected functions of nerves and muscles.

Biochemistry: Decrease in the synthesis of coenzymes such as FMN, FAD, and metabolism of glutathione.

Vertical integration of riboflavin deficiency with para-clinical subjects

General medicine: Study of thymine deficiency-related pathophysiology

Preventive medicine: Prescriptions of food and supplements to prevent riboflavin deficiency.

Vertical integration of riboflavin deficiency with clinical subjects

Gynecology and obstetrics: General health of a woman and additional intake of riboflavin required during pregnancy.

Pediatrics: Correlation of neurological disorders in children with riboflavin deficiency.

NIACIN

Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are coenzymes of niacin that are required for the activation of many enzymes. These coenzymes are synthesized *in vivo* in a series of reactions using tryptophan.

Absorption and Metabolism

After digested food absorption in the small intestine, niacin, nicotinamide, and tryptophan contribute to the synthesis of NAD and NADP. Nicotinamide is deaminated in the body to niacin, which undergoes a series of reactions to produce NAD and NADP.

The RDA, Dietary Sources and Role

BAQ: Discuss the RDA, dietary sources of niacin, and its role in health and disease.

Ans: Dietary sources: Niacin is found in a variety of foods, including liver, chicken, beef, fish, cereal, peanuts, dates, broccoli, carrots, nuts, and legumes, and is also synthesized from tryptophan, which is found in meat, fish, dairy and eggs.

Recommended Dietary Allowance (RDA)

Adults: 15–20 mg per day

Children: 10–15 mg per day

Niacin equivalent = 1 mg niacin = 60 mg tryptophan.

Functions

The functions of a large number of enzymes belonging to the group oxidoreductases are dependent on the coenzymes NAD and NADH. For example, in the following reversible reaction, NAD and NADH both act as coenzymes for the enzyme LDH acting on the substrates pyruvate and lactate, respectively.

$$\text{Pyruvate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NADH}$$

Similarly, NADP is a coenzyme for the enzyme G6PD, in one of the hexose monophosphate shunt reactions, which acts on Glucose 6-P, and the end product is 6-phosphogluconate. HMG-CoA reductase requires NADPH in the conversion of HMG-CoA to mevalonate.

Deficiency, Symptoms, Diagnosis

Niacin deficiency symptoms: A severe deficiency of niacin in the diet causes the disease **pellagra**. It is characterized mainly by diarrhea, dermatitis, and dementia. The symptoms of pellagra are hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue, digestive disturbances, amnesia, and delirium.

Dermatitis (Fig. 11.5) is usually found in areas of skin exposed to sunlight (parts of the face, neck, etc.). Dementia is associated with the degeneration of nervous tissue. Common psychiatric symptoms of niacin deficiency include anxiety, fatigue, poor concentration, irritability, restlessness, apathy, and depression.

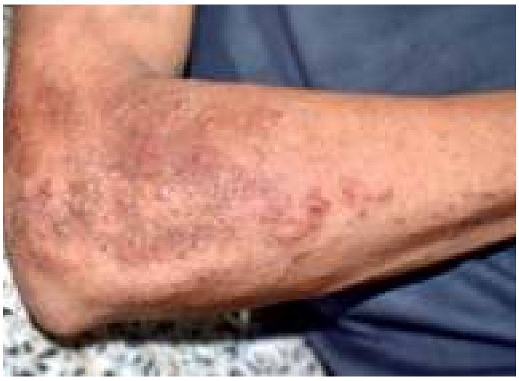


Fig. 11.5: Dermatitis caused by pellagra

Hartnup's disease: Hartnup's disease is a hereditary nutritional disorder resulting in niacin deficiency. It is due to a deficit in the intestines and kidneys which prevents the breakdown and absorption of dietary tryptophan. The resulting condition is similar to pellagra, including symptoms of red, scaly rash, and sensitivity to sunlight. Niacin synthesis is also deficient in carcinoid syndrome, because of the metabolic diversion of tryptophan to form excessive serotonin. Mild niacin deficiency has been shown to slow metabolism, causing decreased tolerance to the cold.

SAQ: Write a note on the toxicity of niacin.

Ans: Pharmacological doses of niacin (1.5–6 g per day) often lead to side effects such as dry skin, itching, skin flushing, and skin rashes. Gastrointestinal symptoms include dyspepsia and liver toxicity. Another side-effect of niacin toxicity is hyperuricemia (increase in blood uric acid), which may lead to gout.

Laboratory Method

SAQ: Give information on laboratory determination of niacin.

Ans: HPLC methods are used to determine urinary metabolites.

Most determinations of niacin have been based on measurement of the urinary metabolites of niacin. Normal excretion of niacin metabolites is 30–60%. Decreased excretion of niacin metabolites indicates niacin deficiency.

Determination of the ratio of NAD/NADH in erythrocytes can be useful to determine niacin deficiency. A ratio of NAD/NADH below 1.0 indicates niacin deficiency.

BAQ: Describe pellagra under the following heads:

1. Symptoms
2. Diagnosis
3. Biochemical basis
4. Treatment.

Ans:

1. **Symptoms:** Diarrhea, dermatitis, hyperpigmentation, thickening of the skin inflammation of the mouth and tongue, and dementia.
2. **Diagnosis:** Consideration of symptoms in physical examination of the patient and by the determination of urinary excretion of niacin metabolites.
3. **Biochemical basis:** Deficiency of niacin leads to deficiency of the coenzymes NAD and NADP, which are required in numerous biochemical processes including glycolysis, metabolism of amino acids, and in the generation of ATP bonds. Decreased synthesis of tryptophan and related metabolites such as melatonin, picolinic acid, and urocanic acid leads to skin photosensitivity. Depression in pellagra is due to serotonin deficiency caused by decreased tryptophan availability to the brain. Anxiety and other neurological disturbances may be caused by 5-aminolevulinic (5-ALA) and the tryptophan metabolite, kynurenic acid.
4. **Treatment:** One pill of vitamin B-complex (containing 10 mg thiamine) with additional food supplements till symptoms are resolved. Thereafter, intake of adequate food supplements.

Horizontal integration of niacin deficiency with pre-clinical subjects

Anatomy:

Skin: Affected skin: Dermatitis

Gastrointestinal tract: Gastrointestinal disturbances

Nervous system: Episodes of dementia, amnesia, delirium, depression

Nerve tissue: Degeneration of nerve tissue

Physiology: Effects on general digestion and absorption of food.

Affected functions of the nervous system

Biochemistry: Affected various metabolic pathways that require NAD and NADH coenzymes. Affected metabolism of tryptophan.

Vertical integration of niacin deficiency with para-clinical subjects

General medicine: Study of niacin deficiency pathophysiology

Preventive medicine: Prescriptions of food and supplements to prevent niacin deficiency.

PANTOTHENIC ACID

Pantothenic acid is required to synthesize coenzyme-A (CoA), which plays an important role in the metabolisms of proteins, carbohydrates, and fats. Since pantothenic acid participates in a wide array of important biological roles, it is essential to all forms of life.

Absorption and Metabolism

BAQ: Write a note on the absorption of pantothenic acid.

Ans: Pantothenic acid is in the form of CoA or acyl carrier protein (ACP) in most foods. For the intestinal cells to absorb this vitamin, it must be converted into free pantothenic acid. CoA and ACP are hydrolyzed within the lumen of the intestine into 4′-phosphopantetheine, which is then dephosphorylated into pantetheine. Pantetheinase (an intestinal enzyme) hydrolyzes pantetheine into free pantothenic acid. Free pantothenic acid is absorbed into intestinal cells through a saturable, sodium-dependent active transport system. At high levels of intake, when this mechanism is saturated, some pantothenic acid is also absorbed by passive diffusion. However, the absorption rate decreases when the concentration of pantothenic acid is high.

Discuss the RDA, Dietary Sources, Role

BAQ: Discuss the RDA, dietary sources of pantothenic acid, its role in health and disease.

Ans: *Dietary sources:* The rich sources of pantothenic acid are eggs, cereals, legumes, meat, yeast, milk, etc.

Recommended Dietary Allowance (RDA):

For children, the RDA of pantothenic acid is 2–4 mg per day.

For adults, pregnant and lactating women RDA is 5–10 mg per day.

Functions

Cells use pantothenic acid for the synthesis of coenzyme A (CoA), which is involved in the metabolism of carbohydrates, proteins, and lipids. Various functions of coenzyme A are as follows:

1. CoA acts as an acyl group carrier to form acetyl-CoA and other related compounds. This is one way to transport carbon atoms within the cell.
2. CoA is important in energy metabolism for pyruvate to enter the tricarboxylic acid cycle (TCA cycle) as acetyl-CoA, and for α-ketoglutarate for the conversion to succinyl-CoA in the cycle.
3. CoA plays an important role in the biosynthesis of many important compounds, such as cholesterol, fatty acids, and acetylcholine.
4. CoA is required in the formation of ACP, which is also required for fatty acid synthesis in addition to CoA.
5. Pantothenic acid in the form of CoA is also required for acylation and acetylation, which are involved in signal transduction and enzyme activation and deactivation, respectively.
6. Calcium pantothenate is often used in dietary supplements because, as a salt, it is more stable than pantothenic acid in the digestive tract, allowing for better absorption.

Pantothenic Acid Deficiency Symptoms

Pantothenic acid deficiency is exceptionally rare. Symptoms of deficiency are due to impaired energy production, due to low

CoA levels. This could cause symptoms of irritability, fatigue, and apathy. Since acetylcholine synthesis is impaired; neurological symptoms can appear in deficiency including burning sensation of feet, numbness, and muscle cramps. Other symptoms include nausea, malaise, restlessness, sleep disturbances, vomiting, and abdominal cramps.

Laboratory Method

BAQ: Give information on the reference range and laboratory method for the determination of serum pantothenic acid.

Ans: The reference range (normal range) for whole blood (or serum) pantothenic acid is 344 to 583 µg/L and for urinary excretion, the reference range is 1–15 mg/day. Urinary excretion of pantothenic acid less than 1 mg/day is considered significantly low.

The methods used for the determination of pantothenic acid are RIA and gas chromatography.

PYRIDOXIN

Pyridoxin (vitamin B_6) is a water-soluble vitamin and is part of the vitamin B complex group. Following seven forms of this vitamin are known: Pyridoxine (PN), the form that is given as a vitamin B_6 supplement, pyridoxine 5′-phosphate (PNP), pyridoxal (PL), pyridoxal 5′-phosphate (PLP, the metabolically active form), pyridoxamine (PM), pyridoxamine 5′-phosphate (PMP) and 4-pyridoxic acid (PA), the catabolite which is excreted in the urine. All forms of vitamin B_6, except PA, can be interconverted. Pyridoxal phosphate (PLP) is the most active form and is a cofactor in many reactions of amino acid metabolism, including transamination, deamination, and decarboxylation. PLP is also necessary for the enzymatic reaction governing the release of glucose from glycogen.

Absorption and Metabolism

Vitamin B_6 in food is absorbed in the jejunum and ileum by passive diffusion. Pyridoxal phosphate and pyridoxamine phosphate in the digestive tract are absorbed by diffusion, which is driven by the trapping of the vitamin by the action of a pyridoxal kinase in the jejunal mucosa. The trapped pyridoxine and pyridoxamine are oxidized to pyridoxal phosphate in the tissue.

The RDA, Dietary Sources, Functions, Role

LAQ: Discuss the RDA, dietary sources of pyridoxin, and its role in health and disease.

Ans: Dietary sources: Milk, fish, meat, eggs, etc. are animal sources rich in vitamin B_6. Good vegetable sources are banana, cabbage, wheat, corn, tubers, and roots.

NOTE

More than 50% of vitamin B_6 is lost by cooking and during storage and processing, depending on the type of vitamin present. Plant foods lose the least during processing as they contain mostly pyridoxine which is far more stable than the pyridoxal or pyridoxamine found in animal foods.

Recommended Dietary Allowance

For adults, the recommended daily allowance is 2.0–2.2 mg per day.

For pregnant and lactating women and the elderly RDA is 2.5 mg per day.

Functions

The primary role of vitamin B_6 is to act as a coenzyme to many other enzymes in the body that are involved in various metabolic reactions. This role is performed by the active form, pyridoxal phosphate. This active form is converted from the two other natural forms found in food: Pyridoxal, pyridoxine, and pyridoxamine. Vitamin B_6 is involved in the following various metabolic processes: Amino acid, glucose, and lipid metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis, and functions and gene expression.

Deficiency, Symptoms, Diagnosis

Vitamin B_6 deficiency Symptoms: Vitamin B_6 deficiency is associated with neurological symptoms such as irritability, depression, nervousness and mental confusion. In severe deficiency, peripheral neuropathy and convulsions are observed due to decreased synthesis of epinephrine, norepinephrine, serotonin, and GABA.

Vitamin B_6 deficiency is associated with **hypochromic microcytic anemia** (Fig. 11.6), with decreased hemoglobin levels, due to a decrease in heme synthesis.

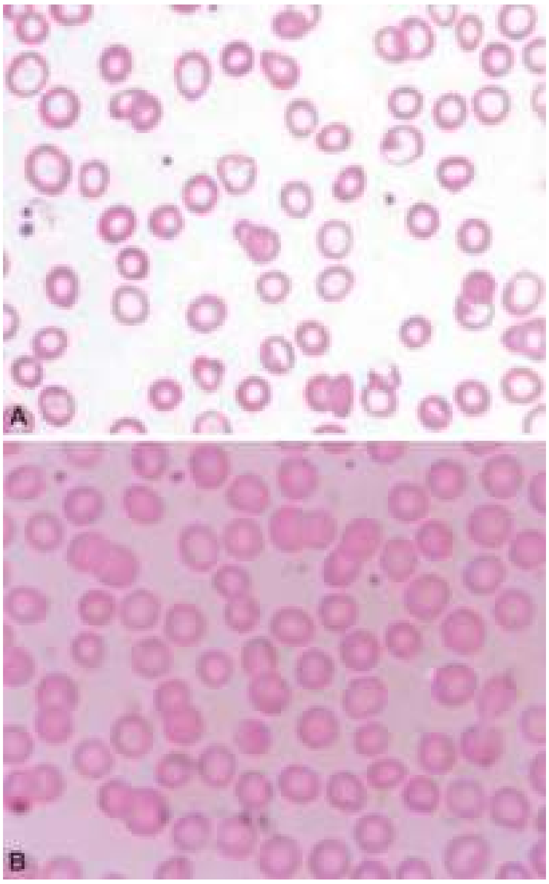


Fig. 11.6.: Stained blood smears: (A) Hypochromic, microcytic RBCs, (B) Normal RBCs

Excretion

The products of vitamin B_6 metabolism are excreted in the urine. The major excretory product is 4-pyridoxic acid. It has been estimated that 40–60% of ingested vitamin B_6 is oxidized to 4-pyridoxic acid. Other products of vitamin B_6 metabolism that are excreted in the urine when high doses of the vitamin include pyridoxine, pyridoxal, pyridoxamine, and their phosphates. A small amount of vitamin B_6 is also excreted in the feces.

Toxicity of Vitamin B_6

Adverse effects of vitamin B_6 have only been documented from vitamin B_6 supplements and not from food sources. Sensory neuropathy has been observed from a high intake of supplemental forms of vitamin B_6. Symptoms of sensory neuropathy include pain and numbness of the extremities, and in severe cases, difficulty in walking. Sensory neuropathy typically develops at doses of pyridoxine over 1,000 mg per day.

Laboratory Method

Q: Give information on reference range and laboratory tests for the determination of pyridoxin.

Ans: Pyridoxal phosphate in the plasma is considered to be one of the best indicators of vitamin B_6 status in the body.

Reference range: 5–50 µg/dl

When plasma pyridoxal phosphate (PLP) is less than 5 µg/dl, it is indicative of vitamin B_6 deficiency.

Low urinary 4-pyridoxic acid (4-PA) is also an indicator of vitamin B_6 deficiency. Urinary 4-pyridoxic of less than 0.8 mg/day is suggestive of vitamin B_6 deficiency.

Determination of serum PLP can be performed by HPLC techniques for the separation of PLP, followed by fluorometric determination.

BAQ: Name two drugs that create pyridoxin deficiency, with reasons.

Ans:

1. Drug-induced vitamin B_6 deficiency can be associated with the therapy of isoniazid, a drug frequently used for treating tuberculosis. Isoniazid combines with pyridoxal phosphate to form inactive hydrazone derivatives.
2. Penicillamine is a drug used to treat patients with rheumatoid arthritis, cystinuria, and Wilson's disease. It reacts

with pyridoxal phosphate to form inactive derivatives and treatment of penicillamine may lead to vitamin B_6 deficiency.

BAQ: Enumerate four important metabolic reactions, which require vitamin B_6.

Ans:

1. Pyridoxal phosphate (PLP) is a cofactor in transaminases that catabolize amino acids. It is involved in almost all amino acid metabolisms (from synthesis to breakdown).
2. Vitamin B_6 plays an important role in gluconeogenesis. Pyridoxal phosphate acts as a cofactor for transaminases to perform transamination reactions that are essential for providing amino acids (pyruvate molecules) as a substrate for gluconeogenesis.
3. Vitamin B_6 acts as a coenzyme for glycogen phosphorylase, the enzyme that is necessary for glycogenolysis.
4. Pyridoxal phosphate aids in the synthesis of heme, and it can bind to two sites on hemoglobin to enhance the oxygen binding of hemoglobin.

SAQ: Enumerate the names of the neurotransmitters that require vitamin B_6.

Ans: Pyridoxal phosphate-dependent enzymes play a role in the biosynthesis of important neurotransmitters such as epinephrine, norepinephrine, serotonin, and gamma-aminobutyric acid.

SAQ: What is the role of vitamin B_6 in the maintenance of normal homocysteine in the body and blood circulation and its clinical significance?

Ans: Pyridoxine (B_6) has a role in the maintenance of normal homocysteine in the body. It acts as a cofactor for the enzyme methionine synthase which plays an important role in the conversion of homocysteine to methionine. Without sufficient pyridoxine, homocysteine builds up in the body. High levels of homocysteine damage blood vessel linings, setting the stage for plaque deposition in arteries. Thus, vitamin B_6 prevents this build-up and decreases the risk of heart disease.

SAQ: Enumerate four examples of the role of pyridoxal phosphate (PLP) in the synthesis of important amines.

Ans: Pyridoxal phosphate (PLP) is used as a coenzyme to create physiologically active amines by decarboxylation of amino acids. Some notable examples of this include histidine to histamine, tryptophan to serotonin, glutamate to gamma-aminobutyric acid (GABA) and dihydroxyphenylalanine to dopamine.

SAQ: Give two examples of the roles of pyridoxal phosphate in the metabolism of important metabolites.

Ans: Synthesis of histamine and coenzyme for enzymes that converts methionine to cysteine.

SAQ: Give two examples of the roles of vitamin B_6 in the synthesis of a phospholipid and one vitamin.

Ans:

1. Vitamin B_6 is an essential component of enzymes that facilitate the biosynthesis of sphingolipids.
2. Vitamin B_6 is also required for the conversion of tryptophan to niacin.

Horizontal integration of vitamin B_6 deficiency with pre-clinical subjects

Anatomy:

- Nervous system: Dysfunction
- Red blood cells: Affected normal development

Physiology: Affected peripheral and autonomic nervous system function.

Biochemistry: Affected metabolic processes: Amino acid, glucose and lipid metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis, and functions and gene expression. Affected metabolic reactions of amino acid metabolism, such as transamination, deamination, and decarboxylation.

Vertical integration of vitamin B_6 deficiency with para-clinical subjects

General medicine: Study of biotin deficiency-related pathophysiology.

Preventive medicine: Study of prescriptions of food and supplements to prevent pyridoxine deficiency.

Vertical Integration of Vitamin B_6 Deficiency with Clinical Subjects

Pediatrics: Consideration of levels of serum pyridoxin in children for the normal development.

Obstetrics and gynecology: Additional supplements containing pyridoxin to the mother to prevent effects on normal fetus growth. Biotin deficiency during pregnancy may cause infants congenital malformations such as cleft palate.

BIOTIN

Biotin is necessary for the production of fatty acids, and the metabolism of fats and amino acids. It plays an important role in the citric acid cycle, by which biochemical energy is generated during aerobic respiration. Biotin plays an important role as a cofactor in various metabolic reactions. Biotin deficiency is rare because intestinal bacteria generally produce biotin above the body's daily requirements.

Absorption and Metabolism

Biotin is present in food as a protein-bound form. Proteolysis by protease is required before absorption. This process assists free biotin release from biocytin and protein-bound biotin. After absorption in the gastrointestinal tract, it is distributed to the body cells.

The RDA, Dietary Sources, Functions and Role

LAQ: Discuss the RDA, dietary sources of biotin and its role in health and disease.

Ans: Dietary sources: The rich sources of biotin are eggs, liver, kidney, milk, tomatoes, grains, etc.

Recommended Dietary Allowance (RDA)

A daily intake of 20–30 µg per day is recommended for adults. Although biotin is synthesized by intestinal bacteria, its availability to the body is not known clearly.

Functions

At the cellular level biotin works as a cofactor responsible for carbon dioxide transfer in several carboxylase enzymes such as acetyl-CoA carboxylase alpha, acetyl-CoA carboxylase beta, methylcrotonyl-CoA carboxylase, phosphoenolpyruvate carboxylase, propionyl-CoA carboxylase, and pyruvate carboxylase. Biotin covalently attaches to the epsilon-amino group of specific lysine residues in these carboxylases. This biotinylation reaction requires ATP and is catalyzed by holocarboxylase synthetase. By working as a cofactor, it plays an important role in fatty acid synthesis, branched-chain amino acid catabolism, and gluconeogenesis.

Deficiency, Symptoms, Diagnosis

Biotin deficiency symptom: Biotin deficiency is rare since it is well distributed in animal and plant foods and also synthesized by intestinal bacteria. However, deficiency may be associated with the following causes:

1. Prolonged use of antibiotic treatment, which destroys intestinal bacterial flora.
2. Excessive consumption of raw egg white. Raw egg white contains avidin, which binds biotin strongly, and thus biotin is not absorbed in the general blood circulation. During cooking, avidin is inactivated and biotin remains intact.

Symptoms of biotin deficiency include hair loss (alopecia), dermatitis in the form of a scaly red rash around the eyes, nose, mouth, and genital area, and conjunctivitis. Neurological symptoms of biotin deficiency include depression, lethargy, hallucination, numbness, and tingling of the extremities.

Biotin deficiency during pregnancy may cause infants congenital malformations such as cleft palate.

Inherited Metabolic Disorders, Leading to biotin Deficiency

BAQ: Write a note on inherited metabolic disorders that lead to biotin deficiency.

Ans: Inherited metabolic disorders are characterized by deficient activities of biotin-dependent carboxylases (multiple carboxylase deficiency). These include deficiencies in the enzyme holocarboxylase synthetase or biotinidase. Holocarboxylase synthetase deficiency prevents the body's cells from using biotin effectively and thus interferes with multiple carboxylase reactions. Biotinidase deficiency is an autosomal recessive metabolic disorder in which biotin is not released from proteins in the diet during digestion or from normal protein turnover in the cell. This situation results in biotin deficiency.

Biotin Toxicity

There are no reported cases of adverse effects from receiving high doses of biotin.

Q: Give information on reference range and laboratory determination of biotin.

Ans: The normal range of biotin in whole blood is 0.5–2.2 nmol/L. Serum values below 0.5 nmol/L indicate biotin deficiency.

Laboratory Method

Laboratory Determination of Biotin

After separation by HPLC methods, ELISA techniques are used for the determination of serum biotin. In the methods based on ELISA, biotinylated primary antibodies are used against serum biotin (as an antigen). It is followed by a detection step using streptavidin conjugated to horseradish peroxidase, followed by the color reaction with a chromogen.

Q: What is the use of biotin in ELISA and RIA tests?

Ans: The attachment of biotin to a specific probe in ELISA and RIA tests is used as an important laboratory technique to study various processes, including protein interactions, protein localization, DNA transcription, replication, etc.

Horizontal integration of biotin deficiency with pre-clinical subjects

Anatomy:

Skin: Hair loss, dermatitis

Nervous system: Dysfunction

Physiology: Peripheral and autonomic nervous system: Affected normal function.

Biochemistry: Metabolic disorders are characterized by deficient activities of biotin-dependent carboxylases (multiple carboxylase deficiency). These include deficiencies in the enzyme holocarboxylase synthetase.

Abnormal increase of 3-hydroxy isovaleric acid. This reflects the reduced status of biotin.

Vertical integration of biotin deficiency with pre-clinical subjects

General medicine: Study of biotin deficiency-related pathophysiology.

Preventive medicine: Study of prescriptions of food and supplements to prevent biotin deficiency.

Vertical Integration of Biotin Deficiency with Clinical Subjects

Pediatrics: Consideration of levels of serum biotin in children with relation to biotin deficiency

Obstetrics and gynecology: Additional supplements containing biotin to the mother to prevent effects on normal fetus growth. Biotin deficiency during pregnancy may cause infants congenital malformations such as cleft palate.

FOLIC ACID

Folic acid consists of a pteridine ring, p-amino benzoic acid (PABA), and glutamic acid. Folic acid is a very important component of tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver. Folic acid is needed for energy production and a strong immune system. It is crucial for the maintenance of every cell in the body, including red and white blood cells, skin cells, and the cells that line the small intestine. Folic acid plays an important role in the synthesis of DNA and RNA. Folic acid is especially important during periods of rapid cell division and growth and production of healthy red blood cells. Folic acid and other micronutrients are used to prevent deformities and diseases, during fetal development.

Absorption and Metabolism

BAQ: Write a note on the absorption and metabolism of folic acid.

Ans: The absorption and metabolism of folic acid in food take place as follows:

1. Dietary folic acid is present as polyglutamate with 3–7 glutamate residues held by peptide bonds. The enzyme folate conjugate in duodenum and jejunum splits the glutamate residue, and only mono glutamate of folic acid is absorbed from the intestine. It is carried to the body cells in general circulation.
2. Within the cells mono glutamate of folic acid is converted to polyglutamate, mainly in the form of tetrahydrofolate (THF), which is actively involved in the one-carbon metabolism.
3. THF binds to one carbon unit at position N^5 or N^{10} (or both) of the pteroyl structure to form folinic acid. Commonly found one carbon moieties formed are N^5-formyl-THF, N^{10}-formyl THF, N^5-formimino-THF, N^5, N^{10}-methenyl-THF, N^5, N^{10}-methylene-THF, and N^5-methyl-THF.

Q: Discuss the RDA, dietary sources of folic acid, and its role in health and disease.

Ans: Dietary sources: The rich sources of folic acid are leafy green vegetables (spinach, asparagus, turnip greens), cereals, whole grains, eggs, liver, kidney, and yeast. In moderate amounts folic acid is also present in certain fruits such as oranges, cantaloupe, honeydew melon, banana, raspberry, grapefruit, and strawberry.

Recommended Daily Allowance (RDA, DFE)

Adults: 400 µg
Children 1–3 years old: 150 µg
Children 4–8 years old: 200 µg
Children 9–15 years old: 300 µg/day
Pregnant women: 600 µg
During lactation: 500 µg/day

NOTE

1. The measure of µg dietary folate equivalents (DFE) is used because the body absorbs more folic acid from fortified foods and dietary supplements than folate found naturally in foods. Compared to folate found naturally in foods, the actual need for folic acid is less to get recommended amounts. For example, 240 µg of folic acid and 400 µg of folate are both equal to 400 µg DFE.
2. Folic acid is a synthetic form present in synthetic foods and supplements. Folate is the natural form present in dietary whole foods.
3. Folate is naturally present in foods such as vegetables (dark green leafy vegetables such as spinach and mustard greens), fruits (oranges), beef liver, nuts (walnuts), beans, and peas.

Functions

All the biological functions of folic acid are performed by tetrahydrofolate and other derivatives. Their biological availability to the body depends upon dihydrofolate reductase action in the liver. Various functions of folic acid are as follows:

1. Folate is necessary for the production and maintenance of new cells, for DNA synthesis and RNA synthesis, and for preventing changes to DNA (cancer prevention action).

2. It is especially important during periods of rapid cell division and growth, such as infancy and pregnancy.
3. Folate is needed to carry one-carbon groups for methylation reactions and nucleic acid synthesis. Thus, folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and neoplasms because of rapid cell division.
4. Both adults and children need folate to make normal red and white blood cells and prevent anemia. Since folate deficiency limits cell division, erythropoiesis and the production of red blood cells are seriously affected and lead to megaloblastic anemia.
5. Deficiency of folate in pregnant women has been implicated in neural tube defects. It has been observed that neural tube defects occur early in pregnancy (first month). Therefore, women are advised to have sufficient folate upon conception.

Folic Acid Deficiency Symptoms

Folate deficiency may lead to diarrhea, glossitis, depression, confusion, megaloblastic anemia, and during pregnancy, fetal neural tube defects and brain defects.

Toxic Effects of Folic Acid

The risk of toxicity from folic acid is low because folate is a water-soluble vitamin and is regularly removed from the body through urine.

Laboratory Method

BAQ: Give information on the reference ranges of serum and erythrocyte folate and laboratory test.

Ans: Reference ranges:

Serum folate: 2.6–12.2 µg/L.

Erythrocyte folate: 103–411 µg/L.

Laboratory Determination of Folic Acid:

Competitive protein binding (CPB) assays are used for the determination of serum or erythrocyte folic acid, followed by RIA or fluorometric techniques. The protein used for binding folate is β-lactoglobulin (milk folate binder) together with a radioactive ^{125}I-folate label or enzyme-linked fluorescent compound.

BAQ: What is the importance of a complete blood count (CBC) test in the diagnosis of folic acid deficiency?

Ans: Folic acid deficiency leads to megaloblastic anemia since folic acid is essential for the normal formation of red blood cells. In the deficiency of folic acid, anemia is indicated when blood hemoglobin is less than 12 g/dl and red blood cell parameters—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) parameters are high. In stained blood smear, large numbers of macrocytes (increased size of RBCs) are detected by microscopic examination (Fig. 11.7).

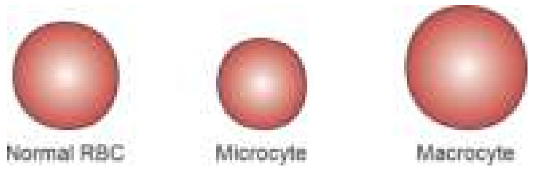


Fig. 11.7: Comparison of a normal RBC with microcyte and macrocyte

BAQ: How folic acid deficiency is diagnosed?

Ans:

1. Complete blood count parameters indicate macrocytic anemia with low blood hemoglobin. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) parameters are high and large numbers of macrocytes (increased size of RBCs) are detected in stained blood smear by microscopic examination (Fig. 11.7).
2. Decrease in an erythrocyte folate level of less than 103 µg/L indicates inadequate folate status.

SAQ: Why erythrocyte folate determination is important to diagnose folic acid deficiency rather than a complete blood count (CBC)?

Ans: Complete blood count may indicate megaloblastic anemia, due to folic acid deficiency but this could also be a sign of vitamin B_{12} deficiency. A serum folate of 2.5 µg/L or lower indicates folic acid deficiency. Serum folate level reflects folate status but erythrocyte folate level better reflects tissue stores after intake.

BAQ: Describe megaloblastic anemia under the following heads:

1. Symptoms
2. Biochemical basis
3. Diagnosis
4. Treatment

Ans:

1. **Symptoms:** Frequent diarrhea, glossitis, headache, lethargy, depression, confusion, pale skin.
2. **Biochemical basis:** Folate is necessary for the production and maintenance of new cells, for DNA synthesis and RNA synthesis, and for preventing changes to DNA. Thus, folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and ultimately the normal development of red blood cells.
3. **Diagnosis:** Complete blood count (CBC) indicates megaloblastic anemia. In CBC, low blood hemoglobin. mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) parameters are high and large numbers of macrocytes (increased size of RBCs) are seen in stained blood smear (Fig. 11.8).

   Serum folate test: A serum folate of 2.5 µg/L or lower indicates folic acid deficiency.
4. **Treatment:** 5 mg folic acid tablet per day, with additional food supplements, till symptoms resolve and improvements are seen in CBC test parameters.

SAQ: Why does a patient suffering from celiac disease have a folic acid deficiency?

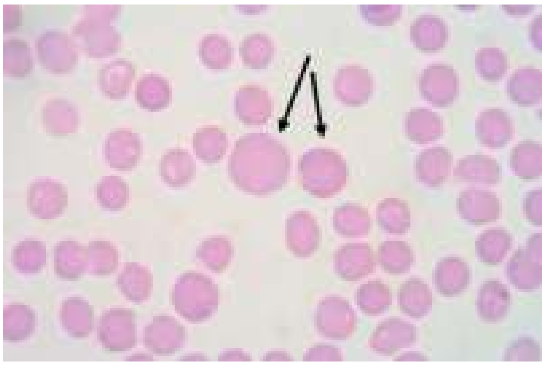


Fig. 11.8: Increased macrocytes in stained blood smear

Ans: Patients suffering from celiac disease have malabsorption problems, which means poor absorption of food ingredients, including micronutrients and vitamins. Hence, patients with celiac disease have a higher chance of developing folate deficiency.

SAQ: How folic acid deficiency is treated?

Ans: Folate deficiency is treated with supplemental oral folate of 400 to 1000 µg per day. This treatment is very successful in replenishing tissues even if the deficiency was caused by malabsorption.

BAQ: Enumerate four important functions of folic acid.

Ans:

1. Folate is necessary for the production and maintenance of new cells, for DNA synthesis and RNA synthesis, and for preventing changes to DNA (cancer prevention action).
2. It is especially important during periods of rapid cell division and growth, such as infancy and pregnancy.
3. Folate is needed to carry one-carbon groups for methylation reactions and nucleic acid synthesis. Thus, folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and neoplasms because of rapid cell division.
4. Both adults and children need folate to make normal red and white blood cells

and prevent anemia. Since folate deficiency limits cell division, erythropoiesis and the production of red blood cells are seriously affected and lead to megaloblastic anemia.

SAQ: What is the clinical significance of folic acid deficiency in pregnant women?

Ans: Deficiency of folate in pregnant women has been implicated in neural tube defects. It has been observed that neural tube defects occur early in pregnancy (first month). Therefore, women are advised to have sufficient folate upon conception.

SAQ: Explain, how deficiency of folic acid may lead to accumulation of homocysteine and its clinical significance.

Ans: Low levels of folate can also lead to homocysteine accumulation as a result of the impairment of methylation, a one-carbon metabolism mechanism. Accumulation of homocysteine may lead to ischemic heart disease.

SAQ: Give a reason for the use of the antifolate drug methotrexate as a part of cancer therapy and additional intake of folate during this treatment.

Ans: Folate is important for cells and tissues that rapidly divide. Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. The antifolate drug methotrexate is often used to treat cancer because it inhibits the production of the active form of tetrahydrofolate (THF) from the inactive dihydrofolate (DHF) and cancer cells are deprived of folic acid for rapid growth.

SAQ: Why folic acid is added to grain products?

Ans: Folic acid is added to grain products in many countries. Because of the difference in bioavailability between supplemented folic acid and the different forms of folate found in food, the dietary folate equivalent (DFE) system was established. 1 DFE is defined as 1 μg of dietary folate or 0.6 μg of folic acid supplement. This is reduced to 0.5 g of folic acid if the supplement is taken on an empty stomach.

Folic acid naturally found in food is susceptible to high heat and UV. It is heat-labile in acidic environments and may be subject to oxidation. Hence, folic acid is added to grain products.

Horizontal integration of folic acid deficiency with pre-clinical subjects

Anatomy: Red blood cells and white blood cells: Affected normal development

Heart: Affected coronary arteries (due to dysfunctional homocysteine metabolism)

Nervous system: Neuronal degeneration and demyelination of the nervous system

Bone marrow: Dysfunction

Fetal cells: Affected normal development

Physiology: Affected functions of red blood in megaloblastic anemia.

Reduced nucleotide and DNA synthesis.

Disturbed erythropoiesis causes immature precursors of erythrocytes to be released in the circulation leading to megaloblastic anemia.

An increase in homocysteine leads to an increase in thrombogenic events.

Loss of memory, confusion, and psychosis.

Biochemistry: Folate is needed to carry one-carbon groups for methylation reactions and nucleic acid synthesis. Thus, folate deficiency hinders DNA synthesis and cell division, affecting hematopoietic cells and neoplasms because of rapid cell division.

Vertical integration of folic acid deficiency with para-clinical subjects

General medicine: Study of folic acid deficiency associated pathophysiology.

Preventive medicine: Prescriptions of food and supplements to prevent folic acid deficiency.

Hematology: Complete blood count determination for the diagnosis of megaloblastic anemia.

Vertical integration of folic acid with clinical subjects

Pediatrics: Consideration of levels of serum folic acid in newborns and children with relation to megaloblastic anemia.

Obstetrics and gynecology: Consideration of folic acid supplements for mothers during pregnancy for their health, normal fetus growth and to prevent neural tube effect during fetus growth.

VITAMIN B_{12} (COBALAMIN)

Vitamin B_{12} is a water-soluble vitamin present in milk, fish, liver, etc, and is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation of the normal functioning of the brain and nervous system. It plays an important role in the formation of blood, fatty acid synthesis, and energy production. As the largest and most structurally complicated vitamin, it can be produced industrially only through bacterial fermentation procedures.

Vitamin B_{12} also consists of a class of chemically-related compounds (Vitamers: Methylcobalamin, cyanocobalamin, and hydroxocobalamin), all of which have vitamin activity. Cyanocobalamin is a synthetic form of vitamin B_{12}. It does not occur in nature but is used in many pharmaceuticals and supplements, and as a food additive, due to its stability and lower cost. A deficiency of vitamin B_{12} leads to pernicious anemia.

Absorption and Metabolism

LAQ: Write a note on the absorption and metabolism of vitamin B_{12}.

Ans: Absorption and metabolism of vitamin B_{12} present in food take place as follows:

1. Protein-bound vitamin B_{12} present in food is digested in the stomach by proteolytic gastric enzymes, which require an acid pH. Free vitamin B_{12} binds to R-proteins to form a B_{12}–R complex. In the stomach, intrinsic factor (IF, a glycoprotein synthesized by gastric parietal cells) is secreted in response to the presence of food, histamine, gastrin, and pentagastrin.
2. In the duodenum, pancreatic proteases digest R-proteins and release B_{12}, which then binds to IF, to form a complex (B_{12}–IF). The binding of vitamin B_{12} with IF is necessary for its absorption since the receptors on the enterocytes in the terminal ileum of the small bowel only recognize the B_{12}–IF complex. Intrinsic factor also protects the vitamin from catabolism by intestinal bacteria. Therefore, absorption of food vitamin B_{12} requires a normally functioning stomach, exocrine secretions of the pancreas, intrinsic factors and normal duodenum. Problems with any one of these organs may lead to a vitamin B_{12} deficiency. Individuals who lack intrinsic factors have a decreased ability to absorb B_{12}. This results in 80–100% excretion of vitamin B_{12} in the feces.
3. The B_{12}–IF complex, after absorption, is transported into the portal circulation. The vitamin is then transferred to transcobalamin II (TC-II–B_{12}, a β globulin), which serves as the plasma transporter.
4. The TC-II–B_{12} complex binds to a specific cell receptor and gets endocytosed. The transcobalamin-II is degraded within a lysosome, and free B_{12} (in the form of methylcobalamin) is finally released into the cytoplasm, where it may be transformed into the proper coenzyme, methylcobalamin by certain cellular enzymes. Excess methylcobalamin is stored in the liver in the form of deoxyadenosyl B_{12}. Most of the B_{12} secreted in the bile is recycled via enterohepatic circulation.
5. Due to the storage of B_{12} in the liver, nutritional deficiency of this vitamin is rare. B_{12} deficiency may arise in a year if initial stores are low and genetic factors are unfavorable.

The RDA, Dietary Sources, Functions, and Role

LAQ: Discuss the RDA, dietary sources of vitamin B_{12}, and its role in health and disease.

Ans: Dietary sources: The rich sources of vitamin B_{12} are milk, fish, liver, kidney, pork, egg, chicken, curd, etc.

Recommended Daily Allowance (RDA)

Adults: About 3 μg

Children: 0.5–1.5 μg

Women during pregnancy and lactation: About 4 μg.

Functions

1. Vitamin B_{12} is normally involved in the metabolism of every cell of the body, especially in DNA synthesis and regulation.
2. It plays an important role in fatty acid synthesis and energy production.
3. Vitamin B_{12} plays a very important role to regenerate folate in the body.
4. Vitamin B_{12} acts as a coenzyme for the action of enzyme homocysteine methyltransferase on substrates homocysteine and N5-methyl-THF to form methionine and THF.

In vitamin B_{12} deficiency, the activity of homocysteine methyltransferase is decreased significantly leading to significantly reduced levels of tetrahydrofolate (THF). Folic acid deficiency (in the form of TFA) leads to reduced nucleotide and DNA synthesis.

Vitamin B_{12} deficiency symptoms: Vitamin B_{12} deficiency leads to pernicious anemia. The following are various vitamin B_{12} deficiency symptoms: Loss of appetite, weakness, shortness of breath, and headaches. Indigestion, diarrhea, numbness, and tingling of fingers and toes (paresthesia). In the advanced stage, the patient suffers from loss of memory, confusion, and psychosis.

BAQ: What are the main biochemical reasons for vitamin B_{12} deficiency?

Ans: Destruction or absence of intrinsic factors leads to vitamin B_{12} deficiency. The binding of vitamin B_{12} with IF is necessary for its absorption in the small intestine; since the receptors on the enterocytes in the terminal ileum of the small bowel only recognize the B_{12}–IF complex. Intrinsic factor also protects the vitamin from catabolism by intestinal bacteria.

The following are various reasons responsible for the destruction or absence of intrinsic factors (IF), which are responsible for vitamin B_{12} deficiency:

1. Insufficient production of hydrochloric acid and IF in elderly persons.
2. Autoimmune destruction of IF
3. Partial or complete gastrectomy
4. Inherited conditions in which either abnormal IF is produced or it is absent.

Toxic effects of vitamin B_{12}: Vitamin B_{12} has extremely low toxicity and even taking it in enormous doses not appear to be harmful to healthy individuals.

BAQ: What is the reason for the neurological symptoms of vitamin B_{12} deficiency?

Ans: Vitamin B_{12} deficiency leads to neuronal degeneration and demyelination of the nervous system. The symptoms include numbness and tingling of fingers and toes (paresthesia). In the advanced stage, the patient suffers from loss of memory, confusion, and psychosis.

The neurological symptoms are due to the accumulation of methylmalonyl-CoA that interferes with myelin sheath formation. Methylmalonyl-CoA acts as a competitive inhibitor of malonyl-CoA in fatty acid synthesis. New types of branched fatty acids are formed due to the substitution of methylmalonyl CoA. These fatty acids disrupt the normal neuronal cell membrane. Impairment in the fatty acid synthesis in the liver and brain also takes place due to the accumulation of propionyl-CoA, which may lead to further neurological complications.

BAQ: Why a person suffering from gastritis may suffer from vitamin B_{12} deficiency?

Ans: Acute gastritis is caused by specific irritants that damage the mucus lining, which protects the stomach mucosa. The irritants include non-steroidal anti-inflammatory drugs (NSAIDs), like aspirin, ibuprofen, etc., excess alcohol and chronic gastritis are caused due to bacterial infections (e.g. *Helicobacter pylori*) and due by autoantibodies to stomach parietal cells, that produce hydrochloric acid and intrinsic factor. Individuals who lack intrinsic factors have a decreased ability to absorb B_{12}, leading to a deficiency of vitamin B_{12}.

SAQ: Enumerate the causes that may lead to the absorption of food vitamin B_{12} and deficiency of vitamin B_{12}.

Ans: Absorption of food vitamin B_{12} requires:

1. A normal functioning stomach
2. Normal exocrine secretions of the pancreas
3. Presence of intrinsic factors, and
4. Normal duodenum functions.

Problems with any one of these organs may lead to a vitamin B_{12} deficiency.

SAQ: Write a note on a deficiency of vitamin B_{12} due to hereditary defects.

Ans: Hereditary defects in the production of the transcobalamins and their receptors may produce functional deficiencies in B_{12} leading to pernicious anemia and infantile megaloblastic anemia, even in many cases with normal blood B_{12} levels.

SAQ: Write a note on vitamin B_{12} which is provided as a supplement.

Ans: Vitamin B_{12} is provided as a supplement in many processed foods, and is also available in vitamin pill form, including multi-vitamins. Vitamin B_{12} can be supplemented in healthy subjects also by liquid, or injection and is available singly or in combination with other supplements. Cyanocobalamin is converted to its active forms, first hydroxocobalamin and then methylcobalamin and adenosylcobalamin in the liver.

SAQ: What is a folate trap or methyl trap?

Ans: Vitamin B_{12} acts as a coenzyme for the action of the enzyme homocysteine methyltransferase on substrates homocysteine and N5-methyl-THF to form methionine and THF. In vitamin B_{12} deficiency (methylcobalamin), the activity of homocysteine methyltransferase is decreased significantly. As a result, the conversion of N5-methyl-THF to tetrahydrofolate is blocked, leading to significantly reduced levels of THF. This is known as methyl trap or folate trap. Hence in vitamin B_{12} deficiency, folic acid deficiency also results leading to reduced nucleotide and DNA synthesis.

BAQ: What are the differences and common features between pernicious anemia and megaloblastic anemia?

Ans: Difference: Pernicious anemia is related to the absence of intrinsic factor (IF) in the stomach, due to autoantibodies that destroy the parietal cell of the stomach. Parietal cells secrete hydrochloric acid and intrinsic factor (IF). The absence of IF leads to vitamin B_{12} deficiency.

Megaloblastic anemia is caused not only due to deficiency of IF but also due to destruction of parietal cells due to medication and bacterial and viral infections, that affect parietal cell functions.

Common feature: In both these types of anemia, vitamin B_{12} deficiency impairs the metabolism of folic acid.

NOTE

The functional deficiency of folic acid disturbs erythropoiesis causing immature precursors of erythrocytes to be released in the circulation leading to megaloblastic anemia.

BAQ: Write three types of drugs, the adverse effects of which may lead to vitamin B_{12} deficiency.

Ans:

1. Excessive alcohol intake lasting longer phase (more than two weeks) can decrease vitamin B_{12} absorption from the gastrointestinal tract.
2. Reduced secretion of gastric acid and pepsin caused by prolonged use of H2 blockers (drugs that reduce acid in the stomach) can reduce the absorption of protein-bound (dietary) vitamin B_{12}, but not of supplemental vitamin B_{12}. Vitamin B_{12} levels should be monitored in people taking high doses of H2 blockers for prolonged periods.
3. Phenytoin, phenobarbital, primidone: These anticonvulsants have been associated with reduced vitamin B_{12} absorption.

SAQ: Why treating cyanide poisoning, a large amount of hydroxocobalamin (5–10 g) is given intravenously?

Ans: For treating cyanide poisoning, sometimes a large amount of hydroxocobalamin (5–10 g) is given intravenously (sometimes in combination with sodium thiosulfate). The mechanism of action is, the hydroxocobalamin hydroxide ligand is displaced by the toxic cyanide ion, and the resulting harmless B_{12} complex is excreted in urine.

Laboratory Determination

SQA: Describe the laboratory test for the determination of vitamin B_{12} and reference range of serum vitamin B_{12}.

Ans: Laboratory tests for the determination of vitamin B_{12}: Competitive protein binding (CPB) and immunometric assays (RIA and ELISA) have been used for the quantitative determination of vitamin B_{12}.

The reference interval (normal range) for serum vitamin B_{12}: 206–678 ng/L.

However, the reference interval of vitamin B_{12} can vary according to the procedures used in the laboratory.

BAQ: Describe pernicious anemia under the following heads:

1. Symptoms
2. Biochemical basis
3. Diagnosis
4. Treatment.

Ans:

1. **Symptoms:** Weakness, shortness of breath, headaches. Indigestion, diarrhea, numbness and tingling of fingers and toes (paresthesia), loss of memory, and confusion.
2. **Biochemical basis:** Vitamin B_{12} deficiency, due to dysfunctional parietal cells of the stomach that secrete intrinsic factors.
3. **Diagnosis:** Complete blood count (CBC) indicates megaloblastic anemia. In CBC, low blood hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) parameters are high and large numbers of macrocytes (increased size of RBCs) are seen in stained blood smear (Fig. 11.8).

   The reference interval (normal range) for serum vitamin B_{12} is 206–678 ng/L. Values less than 206 ng/L, indicate vitamin B_{12} deficiency.
4. **Treatment:** Vitamin B_{12} injection: 500 µg per day, with additional food supplements till symptoms resolve and improvements are seen in CBC test parameters.

Horizontal Integration of Vitamin B_{12} Deficiency with Pre-clinical Subjects

Anatomy:

Stomach: Destruction of gastric mucosa and defect or damage of the parietal cells

Red blood cells: Normal development.

Heart: Affected coronary arteries (due to dysfunctional homocysteine metabolism).

Nervous system: Neuronal degeneration and demyelination of the nervous system.

Bone marrow: Dysfunction.

Physiology: Defect in the absorption of vitamin B_{12}.

- Affected functions of red blood in pernicious anemia
- Affected functions of the stomach in vitamin B_{12} deficiency
- Reduced nucleotide and DNA synthesis, due to folate trap.
- Disturbed erythropoiesis causes immature precursors of erythrocytes to be released in the circulation leading to megaloblastic anemia.
- An increase in homocysteine leads to an increase in anthropogenic events.
- Loss of memory, confusion, and psychosis.

Biochemistry: The accumulation of methylmalonyl-CoA interferes with myelin sheath formation. Methylmalonyl-CoA acts as a competitive inhibitor of malonyl-CoA in fatty acid synthesis. New types of branched fatty acids are formed due to the substitution of methylmalonyl-CoA. These fatty acids disrupt the normal neuronal cell membrane. Impairment in the fatty acid synthesis in the liver and brain also takes place due to the accumulation of propionyl-CoA, which may lead to further neurological complications.

Vertical Integration of Vitamin B_{12} Deficiency with Para-clinical Subjects

General medicine: Study of vitamin B_{12} deficiency associated pathophysiology.

Preventive medicine: Prescriptions of food and supplements to prevent vitamin B_{12} deficiency.

Hematology: Complete blood count determination for the diagnosis of pernicious anemia and megaloblastic anemia.

Vertical integration of vitamin B_{12} deficiency with clinical subjects

Pediatrics: Consideration of levels of serum vitamin B_{12} in newborns and children with relation to pernicious anemia and megaloblastic anemia.

Obstetrics and gynecology: Consideration of vitamin B_{12} supplements for mothers during pregnancy for their own health and normal fetus growth.

OTHER COMPOUNDS WHICH FUNCTION LIKE VITAMINS

SAQ: Enumerate four compounds that function like vitamins.

Ans: There are compounds like choline, inositol, lipoic acid, and para-aminobenzoic acid (PABA), which also act like vitamins and perform many important functions in the body.

LAQ: Discuss the RDA, food sources, and roles of choline, inositol, lipoic acid, and para-aminobenzoic acid (PABA) in health and disease.

Ans:

CHOLINE

Choline is a water-soluble essential nutrient. It is usually grouped within the B-complex vitamins. Choline is a component of phospholipid lecithin. It is involved in membrane structure and lipid transport.

Choline and its metabolites are needed for the following main physiological purposes: Structural integrity and signaling roles for cell membranes, and synthesis of acetylcholine (cholinergic neurotransmission) as a major source for methyl groups. As one of the lipotropic factors (with betaine and methionine), it prevents accumulation of fatty acids in the liver (fatty liver).

Dietary sources: Choline is present in the liver, chicken, cauliflower, spinach, peanuts, and almonds.

The Recommended Daily Allowance (RDA):

Adult men: 550 mg per day

Adult women: 425 mg per day. A higher RDA is required for pregnant and lactating women.

Choline deficiency: Choline deficiency may be responsible for liver disease, atherosclerosis, and possibly neurological disorders. One symptom of choline deficiency is an elevated level of the liver enzyme ALT. It is particularly important for pregnant women to get sufficient choline, since low choline intake may raise the rate of neural tube defects in infants, and may affect the memory of a child.

Supplements: Choline or betaine supplements also may reduce homocysteine. Choline is a necessary source of methyl groups for methyl group transfer. Supplements of lecithin and choline were found to reduce heart disease in laboratory studies.

INOSITOL

Inositol exists in about nine stereoisomers. The most prominent form, widely occurring in nature is myo-inositol. Inositol is a carbohydrate, although it does not belong to a specific type of sugar. It is almost tasteless, with a small amount of sweetness. It is required for the synthesis of phosphatidylinositol, a constituent of cell membranes. Inositol also acts as a lipotropic factor.

Dietary sources: Inositol or its phosphates and associated lipids are found in many foods, in particular, in cereals with high bran content, nuts, beans, and fruit, especially cantaloupe melons and oranges.

The Recommended Daily Allowance (RDA)

Adults: 2-4 g per day.

Inositol and a number of its mono and polyphosphates function as the basis for several signaling and secondary messenger molecules. They are involved in various biological processes, such as insulin signal transduction, cytoskeleton assembly, serotonin activity modulation, cell membrane potential maintenance, breakdown of fats, and decreasing blood cholesterol.

LIPOIC ACID

Lipoic acid (LA) is an organosulfur compound derived from octanoic acid. The precursor to lipoic acid, octanoic acid, is made via fatty acid biosynthesis in the form of octanoyl acyl carrier protein. It is a sulfur-containing fatty acid and exists in an oxidized and reduced form.

Dietary sources: Lipoic acid is found in almost all foods, but mainly in kidney, heart, liver, spinach, broccoli, and yeast extract.

Lipoic acid is involved in the decarboxylation reactions along with other B-complex vitamins. It is required for the conversion of pyruvate to acetyl-CoA and 2-oxoglutarate to succinyl-CoA.

PARA-AMINOBENZOIC ACID (PABA)

Para-aminobenzoic acid (PABA) is an organic compound and consists of a benzene ring substituted with an amino group and a carboxyl group.

Dietary sources: Food sources of PABA include liver, brewer's yeast, kidney, molasses, and whole grains.

The Recommended Daily Allowance (RDA):

Adults: 300–400 mg per day

PABA is an intermediate in the bacterial synthesis of folate. Sulfonamide drugs are structurally similar to PABA, and their antibacterial activity is due to their ability to interfere with the conversion of PABA to folate by the enzyme dihydropteroate synthetase present in microorganisms.

Although any recognized syndromes of PABA deficiency in humans are not observed, many claims of benefit are made by commercial suppliers of PABA as a nutritional supplement. PABA is beneficial in depression, irritability, fatigue, moist eczema, scleroderma (premature hardening of the skin), patchy pigment loss in the skin (vitiligo), and premature grey hair.

Multiple Choice Questions

Q1. Which of the following is the most essential nutrient for a woman during pregnancy to prevent birth defects?

A. Vitamin E
B. Vitamin C
C. Folic acid
D. Vitamin A

Q2. Leading cause of blindness in children is

A. Glaucoma
B. Vitamin A deficiency
C. Vitamin C deficiency
D. Colour blindness

Q3. Good food sources of vitamin C are

A. Broccoli B. Black currants
C. Orange juice D. All of the above

Q4. Which of the following vitamins plays an important role in blood clotting?

A. Vitamin A B. Vitamin K
C. Vitamin D D. Vitamin E

Q5. Vitamin A deficiency may lead to

A. Pellagra
B. Night blindness
C. Pernicious anemia
D. Rickets

Q6. One important function of vitamin A is

A. To play an important role in bone formation
B. To act as a coenzyme for some enzymes
C. To maintain the integrity of epithelial tissue
D. To maintain acid–base balance

Q7. Retinal is a component of

A. Rhodopsin
B. Glycoproteins
C. Cardiolipin
D. Lipoprotein

Q8. On exposure to light rhodopsin forms

A. *Cis*-retinal
B. Retinol
C. All *trans*-retinal
D. Retinoic acid

Q9. Vitamins C deficiency may develop in the case of

A. A pregnant woman
B. A malnourished child
C. A long-time alcoholic
D. A, B, C

Q10. Which of the following vitamins functions as both, hormone and visual pigment?

A. Thiamine B. Retinal
C. Riboflavin D. Folic acid

Q11. Megaloblastic anemia is caused by

A. Folic acid
B. Niacin
C. Pyridoxine
D. None of these

Q12. Which of the following is not a fat-soluble vitamin?

A. Vitamin A B. Vitamin D
C. Vitamin B_{12} D. Vitamin K

Q13. Disease that is caused by the deficiency of niacin is

A. Pellagra B. Rickets
C. Scurvy D. Pernicious anemia

Q14. The richest source of vitamin D is

A. Cheese B. Fish liver oils
C. Egg yolk D. Butter

Q15. Deficiency of vitamin D causes

A. Xerophthalmia
B. Hypothyroidism
C. Rickets
D. Osteomalacia
E. C and D

Q16. Calcitriol synthesis takes place in

A. Kidney B. Muscle
C. Adipose tissue D. Intestine

Q17. Which of the following vitamins functions as a coenzyme?

A. Vitamin D
B. Vitamin E
C. Vitamin B_6
D. All of the above

Q18. Tocopherol may be destroyed by

A. Storage in the refrigerator
B. Cooking
C. Storage at room temperature average 25°C
D. A and B

Q19. The most important natural antioxidant is

A. Vitamin K B. Vitamin E
C. Vitamin B_{12} D. Vitamin D

Q20. Tocopherols prevent the oxidation of

A. Vitamin D B. Vitamin A
C. Vitamin C D. Thiamine

Q21. This vitamin prevents the peroxidation of unsaturated fatty acids

A. D B. K C. A D. E

Q22. Important sources of Vitamin K are

A. Liver
B. Cheese
C. Green leafy vegetables
D. All of the above

Q23. The vitamin synthesized by bacteria in the intestine is

A. Pyridoxine B. A
C. K D. C

Q24. Vitamin K is involved in post-translational modification of the blood clotting factors by acting as a cofactor for the enzyme:

A. Deaminase
B. Hydroxylase
C. Decarboxylase
D. Carboxylase

Q25. Hypervitaminosis K in neonates may lead to

A. Jaundice
B. Beriberi
C. Pellagra
D. Xerophthalmia

Q26. One scientific name for vitamin K is

A. Ascorbic acid
B. Pantothenic acid
C. Phytonadione
D. Tocopherol

Q27. Which of the following vitamins is required for the formation of hydroxyproline in collagen?

A. Vitamin A B. Vitamin D
C. Thiamine D. Vitamin C

Q28. Which of the following vitamins is not present in sterilized milk?

A. Vitamin C B. Vitamin A
C. Vitamin D D. Vitamin E

Q29. Scurvy is caused due to the deficiency of

A. Thiamine B. Vitamin C
C. Vitamin A D. Vitamin D

Q30. Wernicke's disease and beriberi can be treated by

A. Vitamin C B. Thiamine
C. Riboflavin D. Vitamin B_{12}

Q31. Thiamine deficiency causes

A. Scurvy
B. Xerophthalmia
C. Rickets
D. Beriberi

Q32. Concentration of pyruvic acid and lactic acid in the blood is increased due to a deficiency of which of the following vitamins?

A. Riboflavin B. Biotin
C. Thiamine D. Vitamin C

Q33. Coenzyme TPP is involved in

A. Deamination
B. Transamination
C. Hydroxylation
D. Decarboxylation of pyruvate

Q34. Increased glucose consumption increases the dietary requirement for

A. Vitamin D B. Niacin
C. Thiamine D. Pantothenic acid

Q35. Riboflavin deficiency causes

A. Pellagra B. Dermatitis
C. Cheilosis D. Loss of weight

Q36. Magenta tongue is found in the deficiency of the vitamin

A. Riboflavin
B. Pyridoxine
C. Nicotinic acid
D. Thiamine

Q37. Corneal vascularization is found in a deficiency of the vitamin

A. Vitamin A B. Riboflavin
C. Vitamin C D. Niacin

Q38. Pellagra is caused due to the deficiency of

A. Riboflavin B. Vitamin C
C. Niacin D. Thiamine

Q39. In the body 1 mg of niacin can be produced from

A. 30 mg of tryptophan
B. 6 mg of tryptophan
C. 60 mg of tryptophan
D. 60 mg of pyridoxin

Q40. Pellagra may occur due to a diet rich in

A. Wheat B. Rice
C. Maize D. Rice

Q41. Nicotinamide acts as a coenzyme for

A. Decarboxylases B. Dehydrogenases
C. Transaminases D. Carboxylases

Q42. Dietary requirement of Vitamin D in adults is about

A. 100 IU B. 150 IU
C. 400 IU D. 200 IU

Q43. Coenzyme A is involved in

A. Transamination B. Dehydrogenation
C. Acetylation D. Decarboxylation

Q44. The precursor of CoA is

A. Pantothenic acid B. Biotin
C. Riboflavin D. Pyridoxine

Q45. Deficiency of which of the following vitamins leads to 'burning foot syndrome'?

A. Thiamine B. Pantothenic acid
C. Biotin D. Pyridoxine

Q46. Pyridoxal phosphate acts as a cofactor in

A. Deamination
B. Carboxylation
C. Transamination
D. Oxidation–reduction

Q47. Vitamin B_6 deficiency may occur during therapy with

A. Paracetamol
B. Aspirin
C. Sulfa drugs
D. Isoniazid

Q48. Deficiency of vitamin B_6 may occur in

A. Diabetes mellitus
B. Alcoholics
C. Hepatitis
D. Both A and B

Q49. Epileptic convulsions in human infants have been attributed to the deficiency of the

A. Vitamin B_{12}
B. Pantothenic acid
C. Pyridoxine
D. Vitamin C

Q50. Biotin is a coenzyme of the enzyme

A. Decarboxylase
B. Peroxidase
C. Carboxylase
D. Hydroxylase

Q51. Consumption of raw eggs can cause a deficiency of

A. Riboflavin
B. Thiamine
C. Biotin
D. Pantothenic acid

Q52. A cofactor required in the oxidative decarboxylation of pyruvate is

A. Thimine
B. PABA
C. Lipoic acid
D. Pyridoxine

Q53. A deficiency of vitamin B_{12} causes

A. Hemolytic anemia
B. Thalassemia
C. Perniciuos anemia
D. Beriberi

Q54. Vitamin B_{12} deficiency can be diagnosed by urinary excretion of

A. Pyruvic acid
B. Lactic acid
C. Methylmalonate
D. Acetone

Q55. Both folic acid and vitamin B_{12} are required in

A. Deamination of serine
B. Methylation of homocysteine to methionine
C. Conversion of pyridoxal phosphate to pyridoxamine phosphate
D. Decarboxylation reactions

Q56. Folate as a coenzyme is involved in the transfer and utilization of

A. Amido group
B. Single carbon moiety
C. Carboxyl group
D. Amino group

Q57. Folic acid deficiency can be diagnosed by increased urinary excretion of

A. Formiminoglutamic acid (Figlu)
B. Uric acid
C. Cystathionine
D. Glutamic acid

Q58. Sulfa drugs interfere with the bacterial synthesis of

A. Thiamine
B. Vitamin B_{12}
C. Tetrahydrofolate
D. Riboflavin

Q59. Folate deficiency causes

A. Iron deficiency anemia
B. Hemolytic anemia
C. Microcytic anemia
D. Megaloblastic anemia

Q60. Thiamine deficiency leads to

A. Beriberi
B. Fatigue
C. Mental depression
D. A, B and C

Q61. Daily requirement of thiamine is

A. 1.0–1.5 mg
B. 5–10 mg
C. 10–50 mg
D. 0.1–0.8 mg

Q62. Thiamine requirement is greater in

A. Gout
B. Nonvegetarians
C. Diabetes mellitus
D. Alcoholics

Q63. Which one of the following is pellagra preventing vitamin?

A. Niacin
B. Riboflavin
C. Vitamin B_6
D. Pyridoxine

Q64. Daily requirement of niacin is about

A. 2.0–5.0 mg
B. 1.0–2.0 mg
C. 150–200 mg
D. 15–20.0 mg

Q65. Deficiency of niacin occurs in

A. Pellagra
B. Pernicious anemia
C. Hartnup disease
D. Scurvy

Q66. Deficiency of pantothenic acid in human beings may affect

A. Digestive system
B. Nervous system
C. Bones
D. Both A and B

Q67. This vitamin used in the treatment of homocystinuria is

A. Pyridoxin B. Riboflavin
C. Pantothenic acid D. Folic acid

Q68. A six-year-old boy was presented with bow legs and a deformed chest. What was the expected vitamin deficiency in this case

A. Vitamin A B. Vitamin C
C. Vitamin D D. Folic acid

Q69. A 19-year-old male patient was presented with a 4-month history of nyctalopia. Ocular findings at presentation included mild xerophthalmic features and nonspecific pigmentary retinal changes. He was probably suffering from a deficiency of

A. Vitamin A B. Vitamin C
C. Vitamin D D. Vitamin B_6

Q70. A 36-year-old alcoholic person presented with depression, nervousness, and mental confusion. He was deficient of vitamins

A. B_1, B_6, B_{12} B. A, D, E
C. B2, D, C D. C, B_7, B_9

Answers

1. C **2.** B **3.** D **4.** B **5.** B **6.** C **7.** A **8.** C **9.** D **10.** B
11. A **12.** C **13.** A **14.** B **15.** D **16.** A **17.** C **18.** B **19.** B **20.** D
21. D **22.** C **23.** C **24.** D **25.** A **26.** C **27.** D **28.** B **29.** B **30.** B
31. D **32.** C **33.** D **34.** C **35.** C **36.** A **37.** B **38.** C **39.** C **40.** C
41. B **42.** C **43.** C **44.** A **45.** B **46.** C **47.** D **48.** B **49.** C **50.** C
51. C **52.** C **53.** C **54.** C **55.** B **56.** B **57.** A **58.** C **59.** D **60.** D
61. A **62.** D **63.** C **64.** D **65.** A **66.** D **67.** A

68. C: Rickets. Presentation with bow legs and a deformed chest.

69. A: Vitamin A deficiency. 4-month history of nyctalopia, which means a clinical condition characterized by an abnormal inability to see in dim light or at night

70. A: Alcoholics suffer from vitamins B_1, B_6, and B_{12} deficiency and related symptoms such as depression, nervousness, and mental confusion.

Case Studies

Case 1: A 72-year-old man complained of difficulty in walking with the sensation of pricking and numbness in his legs. The physical signs indicated neuropathy. Following blood test report indicated thiamine deficiency. Vitamin B complex supplements improved the patient's symptoms.

	Reference range (Normal range)
Blood thymine pyrophosphate: 45 nmol/L	90–140 nmol/L

Q1. What is the biochemical basis for the symptoms seen in this case?

Ans: The accumulation of methylmalonyl-CoA in thiamine deficiency, interferes in myelin sheath formation. Methylmalonyl-CoA acts as a competitive inhibitor of malonyl-CoA in fatty acid synthesis. New types of branched fatty acids are formed due to the substitution of methylmalonyl-CoA. These fatty acids disrupt the normal neuronal cell membrane. Impairment in the fatty acid synthesis in the liver and brain also takes place due to the accumulation of propionyl-CoA, which leads to further neurological complications.

Q2. What are the RDA and sources of thiamine?

Ans: Recommended dietary allowance (RDA):
Adults: 1–1.5 mg per day

Dietary sources: Nuts, cereals, pulses, oils seed, yeast, milk, pork, liver, heart, kidney, etc. It is concentrated in the outer layer of cereals.

Q3. What is the treatment to cure thiamine deficiency in this case?

Ans: Intravenous (IV) or orally (PO): 50 mg thiamine three times daily until symptoms resolve. Later on 10 mg/day oral tablets of thiamine until expected recovery is complete with additional vitamin supplements.

Case 2: An 8-year-old boy was referred to a physician since he was having difficulty seeing in dim light. It was found out that he was also suffering from hyperkeratotic skin lesions, and Bitot's spots were seen in certain parts of the conjunctiva. These symptoms indicated vitamin A deficiency and the following blood test report confirmed the diagnosis. Additional tests were required to find out whether the deficiency of vitamin A was primary or secondary.

	Normal range
Serum β carotene 4.0 μg/dl	10–85 μg/dl

Q1. What is the biochemical basis for the symptoms seen in this case?

Ans: Changed normal chemical nature of corneal epithelial tissue due to deficiency of vitamin A. Deficiency of vitamin A results in corneal epithelial tissue keratinization, ulceration, and necrosis. Vitamin A is necessary for the normal differentiation of non-squamous epithelial tissue and the prevention of excess keratinization of epithelial tissue leading to an effect on vision.

Q2. What are the RDA and sources of vitamin A?

Ans: Recommended dietary allowance (RDA), per day:

Adults:

Male: 900 μg RAE

Sources: Animal sources: Milk, egg yolk, liver, kidney, butter, cheese, fish (shark or cod liver oil), etc.

Vegetable sources (provitamin A): Yellow and dark green vegetables and fruits, e.g. papaya, carrots, spinach, mango, pumpkins, etc.

Q3. What is the treatment to cure vitamin A deficiency?

Ans: 1 dose of 100,000 IU of vitamin A and in age 12–36 months of age one dose of 200,000 IU of vitamin A every six months, with additional vitamin supplements.

Case 3: A 50-year-old man was undergoing anticonvulsant therapy (Drug: Phenobarbital) for several months. He complained of weakness, bone pain, and pain in the hip. His blood test reports were as follows:

	Normal range
Serum calcium: 8.3 mg/dl	9–11 mg/dl
Serum inorganic phosphorus: 1.0 mg/dl	2.5–4.5 mg/dl
Serum alkaline phosphatase: 140 IU	20–80 mg/dl
Serum parathyroid hormone (PTH): 55 ng/L	10–65 ng/L
Serum 25-hydroxy-vitamin D: 3.5 ng/ml	10–65 ng/ml

Q1. What is the diagnosis?

Ans: He is suffering from vitamin D deficiency.

Q2. What is the biochemical basis of this case?

Ans: Chronic anticonvulsant therapy may cause osteomalacia or rickets. Phenobarbital changes the kinetics of 25-hydroxy-vitamin D and stimulates the excretion of bile, leading to decreased concentration of serum 25-hydroxy-vitamin D. It also inhibits intestinal calcium transport and bone mineral mobilization. Serum PTH levels were on the higher side of the normal range due to the decreased intestinal absorption of calcium. Hence serum inorganic phosphorus values are very low compared to serum calcium.

Q3. What is the first line of treatment?

Ans: Appropriate monitoring of the above-listed blood tests with adequate supplements of vitamin D.

Case 4: During a routine examination, an alcoholic 47-year-old man reported a loss of weight, anorexia, headache, and pale yellow skin. His blood test reports were as follows:

	Reference range (Normal range)
Hemoglobin: 9.6 g/dl	13–17 g/dl
Erythrocyte count: 3.9×10^{12}/L	4.5–6.0 × 10^{12}/L
Leucocyte count: 5.5×10^{9}/L	5.0–10 × 10^{9}/L
PCV: 33%	42–52%
MCV: 120 fl	81–92 fl
MCH: 36 pg	27–32 pg
MCHC: 35%	32–36%
Erythrocyte folic acid: 65 µg/L	103–411 µg/L

Q1. What is the diagnosis?

Ans: Megaloblastic anemia. Very low hemoglobin, increased MCV, and MCH, indicate megaloblastic anemia, due to alcoholism.

Q2. What is the biochemical basis?

Ans: Poor absorption of folic acid due to excess alcohol intake.

Q3. What is the treatment?

Ans: Abstinence from alcohol and treatment: 5 mg folic acid tablet per day, with adequate supplements till symptoms are resolved and improvements are seen in CBC test parameters.

Chapter 12

Xenobiotics

SPQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer questions: Marks: 9 or 10

MCQs: Marks: 0.5 each

> *Competency achievement:* The student should be able to:
>
> **BI7.5:** Describe the role of xenobiotics in disease

INTRODUCTION

The term xenobiotics is derived from the Greek words, *xenos* means foreigner (or stranger) and *bios* means life. Xenobiotic is a chemical found in an organism, which is normally not produced in it. Examples of xenobiotics are drugs, food additives, pollutants, etc. Drugs such as antibiotics are xenobiotics since these are not synthesized by the cells.

SAQ: Define xenobiotics.

Ans: Xenobiotic is a chemical found in an organism, which is normally not produced in it.

SAQ: Give three examples of xenobiotics.

Ans:

1. Drugs such as antibiotic
2. Pollutants such as dioxin
3. Food additives such as potassium sorbate

DETOXIFICATION OF XENOBIOTICS

SAQ: How body removes xenobiotics?

Ans: The body removes xenobiotics by specific metabolic reactions. This consists of the deactivation and the secretion of xenobiotics, and these processes mostly take place in the liver. Secretion routes of excretion of end products are urine, feces, breath, and sweat.

SAQ: What is the mechanism of xenobiotic excretion?

Ans: Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them by oxidation, reduction, hydrolysis, or hydration of the xenobiotic, and then conjugating the active secondary metabolite with glucuronic or sulfuric acid, or glutathione, followed by excretion in bile or urine.

SAQ: Give three examples of enzymes involved in xenobiotic metabolism.

Ans:

1. Hepatic microsomal cytochrome P450.
2. Glutathione transferase
3. Epoxide hydrolase

BAQ: Briefly describe three phases of excretion of xenobiotics.

Ans: Xenobiotic metabolism is divided into the following three phases:

- In phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics.
- In phase II, the modified compounds in phase I are then conjugated to polar compounds. These reactions are catalyzed by transferase enzymes such as glutathione S-transferases.
- Finally, in phase III, the conjugated xenobiotics are further converted to metabolites and then excreted in urine by the kidneys.

SAQ: What are cytochrome P450s?

Ans: Cytochrome P450s are the heme-containing enzymes present in cytochrome 450. They are present in most of the tissues. These are present in higher amounts in liver cells and enterocytes in the endoplasmic reticulum.

BAQ: Briefly describe any three phase II reactions of the metabolism of xenobiotics.

Ans:

1. Glucuronidation: Molecules such as aniline, benzoic acid, steroids, 2-acetylaminofluorene (a carcinogen), bilirubin, etc. undergo glucuronidation (conjugation) by the catalytic action of a variety of glucuronyltransferases present in the cytosol and endoplasmic reticulum.
2. Sulfonation: Phenols, arylamines, and some alcohol get sulfonated. The sulfur donor used in these reactions is adenosine 3′-phosphate 5′-phosphosulfate (PAPS).
3. Glutathione conjugation: A large number of potentially toxic electrophilic carcinogens get conjugated to nucleophilic glutathione (GSH) by the catalytic action of glutathione S-transferase as follows:

   $R + GSH \rightarrow R\text{–}S\text{–}G$ ("R" is an electrophilic xenobiotic)

SAQ: Give one example of phase III reactions of the metabolism of xenobiotics.

Ans: A common example of phase III is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates. In this case, the γ-glutamate and glycine residues in the glutathione molecule are removed by gamma-glutamyl transpeptidase and dipeptidases. In the final step, the cysteine residue in the conjugate is acetylated. The conjugates are excreted in urine with the help of a variety of membrane transporters.

LAQ: Describe the role of xenobiotics in disease.

Ans: Xenobiotic is a chemical found in an organism, which is normally not produced in it. Examples of xenobiotics are drugs, food additives, pollutants, etc. Drugs such as antibiotics are xenobiotics since these are not synthesized by the cells.

Xenobiotic metabolism is the set of metabolic pathways that modify the chemical structure of xenobiotics. These pathways are a form of biotransformation present in the human body. The body removes xenobiotics by specific metabolic reactions. This consists of the deactivation and the secretion of xenobiotics, and these processes mostly take place in the liver. Secretion routes of excretion of end products are urine, feces, breath, and sweat.

Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them by oxidation, reduction, hydrolysis, or hydration of the xenobiotic, and then conjugating the active secondary metabolite with glucuronic or sulfuric acid, or glutathione, followed by excretion in bile or urine. An example of a group of enzymes involved in xenobiotic metabolism is hepatic microsomal cytochrome P450.

Xenobiotic metabolism pathways often act to detoxify poisonous compounds. However, in some cases, the intermediates in xenobiotic metabolism can themselves be the cause of toxic effects. One example is the pathogenesis of non-alcoholic fatty liver disease. Genetic background, dietary factors, gut microbiota, and other factors act together in the initiation and progression of non-alcoholic fatty liver disease.

Recent studies have suggested a link between pharmaceutical xenobiotic and non-pharmaceutical chemicals exposure and the initiation and progression of the clinical course of non-alcoholic fatty liver disease. The liver is the major organ for the metabolism of environmental biochemicals and drugs. It has been seen by scientific studies that exposure to some of the xenobiotics may lead to lipid accumulation in hepatocytes through insulin resistance, modulation of nuclear receptor activation, increased fatty acid biosynthesis, mitochondrial dysfunction, and impaired lipid excretion. The progression from simple infiltration of fats in liver cells may progress to steatohepatitis, which means liver inflammation and damage caused by the accumulation of fat in the liver. The factors responsible for steatohepatitis are inflammatory reactions, oxidative stress, and endoplasmic reticulum (ER) stress. These factors ultimately may lead to cell death and cirrhosis of the liver.

Phases of Detoxification of Xenobiotics

LAQ: Write a note on the phases of detoxification of xenobiotics.

Ans: The detoxification of xenobiotics is divided into three phases: Modification, conjugation, and excretion. These reactions act in sequence to detoxify xenobiotics and remove them from cells and tissues. Xenobiotic metabolism takes place in the following three phases:

Phase I: In phase I, a variety of enzymes acts to introduce reactive and polar groups into their substrates. One common example is modifications of xenobiotic compounds by hydroxylation catalysed by the cytochrome P-450-dependent oxidase system. These enzyme complexes act to incorporate an atom of oxygen into nonactivated hydrocarbons, which can result in either the introduction of hydroxyl groups or N^-, O^- and S-dealkylation of substrates. The reaction mechanism of the P-450 oxidases proceeds through the reduction of cytochrome-bound oxygen and the generation of a highly-reactive oxyferryl species, according to the following procedures:

- Hydroxylation: This is the main reaction involved in phase l, and the enzymes which act on xenobiotics are called mono-oxygenases (cytochrome 450s). These enzymes catalyze the following reaction:
  $RH + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP$
- RH in the above reaction represents a variety of xenobiotics such as carcinogens, drugs, pesticides, pollutants and petroleum products. Cytochrome 450 undergoes reduction in this process and it is oxidized back when it reacts with oxygen. More than 50% of drugs and pollutants are detoxicated by cytochrome 450s by the reaction mentioned in hydroxylation.
- Oxidation: A large number of xenobiotics such as aldehydes, alcohols, amines, aromatic hydrocarbons and sulfur compounds are detoxicated by oxidation. Methanol, ethanol and benzoic acid are converted to formic acid, acetic acid, and hippuric acid respectively and excreted by the kidneys. Aldehydes and aliphatic amines are converted to respective corresponding acids and excreted out of the body in urine.

Phase II: In the phase II reactions, the activated xenobiotic metabolites are conjugated with charged compounds such as glucuronic acid, glutathione (GSH), glycine, or sulfate. These reactions are catalyzed by the tranferase enzymes which in combination with organic compounds are able to metabolise many hydrophobic compounds that contain nucleophilic or electrophilic groups. One example of transferases enzymes is the glutathione S-transferases (GSTs). The addition of large anionic groups (such as GSH) detoxifies reactive electrophilic compounds and produces more polar metabolites that cannot diffuse across membranes, and later on are transported actively. Following are the various types of phase II reactions:

- *Glucuronidation:* Molecules such as aniline, benzoic acid, steroids, 2-acetylaminofluorene (a carcinogen), bilirubin, etc. undergo glucuronidation (conjugation) by the catalytic action of variety of glucuronyltransferases present in cytosol and endoplasmic reticulum.
- *Sulfonation:* Phenols, arylamines, and some alcohol are sulfonated. The sulfur donor used in these reactions is adenosine 3′-phosphate-5′-phosphosulfate (PAPS).
- Glutathione conjugation: A large number of potentially toxic electrophilic carcinogens are conjugated to nucleophilic glutathione (GSH) by catalytic action of glutathione S-transferase as follows:

  R + GSH → R–S-G ("R" is an electrophilic xenobiotic)
- *Acetylation:* Some xenobiotics (such as isoniazid) acetylates as follows, by the catalytic action of the enzymes acetyl transferases present in the cytosol of various tissues, particularly liver:

  X + Acetyl-CoA → Acetyl-X + CoA
- *Methylation:* Some xenobiotics are subject to methylation, which is catalyzed by the enzymes, methyltransferases, that require S-adenosylmethionine as a methyl donor.

Phase III: After phase II reactions, the xenobiotic conjugates are further metabolized. A common example is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates. In this case the γ-glutamate and glycine residues in the glutathione molecule are removed by gamma-glutamyl transpeptidase and dipeptidases. In the final step, the cystine residue in the conjugate is acetylated. The conjugates and their metabolic products are excreted from cells with the anionic groups due to their affinity for a large number of cell membrane transporters. These transporters belong to the family of ATP-binding transporters and catalyse the ATP dependent transport of a large number of hydrophobic anions, and remove phase II products to the extracellular medium, where they are further metabolized or excreted.

BAQ: Enumerate the specific features of xenobiotic metabolism.

Ans: The following are the important feature of xenobiotic metabolism:

1. The activities of xenobiotic-metabolizing enzymes are affected by sex, age and specific genetic factors.
2. Metabolites of certain xenobiotics can stimulate or inhibit the activities of xenobiotic-metabolizing enzymes. This can affect the doses of certain drugs that are administered to the patients.
3. Various diseases (e.g. diseases of liver), can affect the activities of drug-metabolizing enzymes.
4. The covalent binding to cell macromolecules by the reactive species (ROS) of xenobiotics produced in metabolic reactions can injure the cells. The micromolecule targets may be DNA, RNA or any protein. Mutations involved in such reaction may lead to initiation of cancer.
5. The reactive species of xenobiotics may behave like a hapten and combine with a specific protein stimulating antibody production, leading to cellular damage.
6. Epoxides are highly reactive and mutagenic products produced by the action of some mono-oxygenases on certain procarcinogenic metabolites involving xenobiotics. Epoxy hydrolase present in the membrane of the endoplasmic reticulum acts on epoxides and converts them into much less reactive dihydrophenols.
7. Enzyme polymorphisms may affect drug metabolism leading to certain pathological conditions such as hemolytic anemia (in G6PD deficiency) following antimalarial treatment and malignant hyperthermia (in mutated calcium release channels) following administration of certain anesthetics.

Another example of interference of xenobiotic metabolic product is the organic compound bisphenol A (BPA). BPA is an organic, synthetic chemical that has been used in the manufacturing of plastics. It has been used in the manufacturing of plastic water bottles and baby bottles as well as in drinking plastic water pipes. BPA competes with estrogen. With the result, estrogen fails to bind to its specific cell membrane receptor and perform normal functions, such as regulation of growth, development, and physiology of the reproductive system, and regulation of adipose, skeletal, cardiovascular systems, and neuroendocrine systems, etc.

Multiple Choice Questions

Q1. Which one of the following is an example of xenobiotic?

A. Glucose B. Glycine
C. Palmitic acid D. Ethanol

Q2. Which one of the following coenzymes plays an important role in the reaction mechanism of cytochrome P450?

A. NAD B. NADH
C. FAD D. NADPH

Q3. Glutathione synthesis does not require

A. Glutamic acid B. Alanine
C. Glycine D. Cysteine

Q4. All cytochrome P450 are

A. Lipoproteins
B. Nucleoproteins
C. Hemoproteins
D. Glycoproteins

Q5. Chief reaction involved in phase I metabolism of xenobiotics is

A. Sulfonation
B. Hydroxylation
C. Acetylation
D. Methylation

Q6. Main enzymes involved in the phase II metabolism of xenobiotics are

A. GSTs
B. CK
C. GPT
D. Both B and C

Q7. Enzymes involved in xenobiotics metabolism are mainly present in

A. Kidneys B. Liver
C. Lungs D. Brain

Q8. Molecules such as benzoic acids, bilirubin and steroids are eliminated from the body by

A. Glucuronidation
B. Sulfonation
C. Acetylation
D. Methylation

Q9. Some xenobiotics like isoniazid are eliminated from the body by

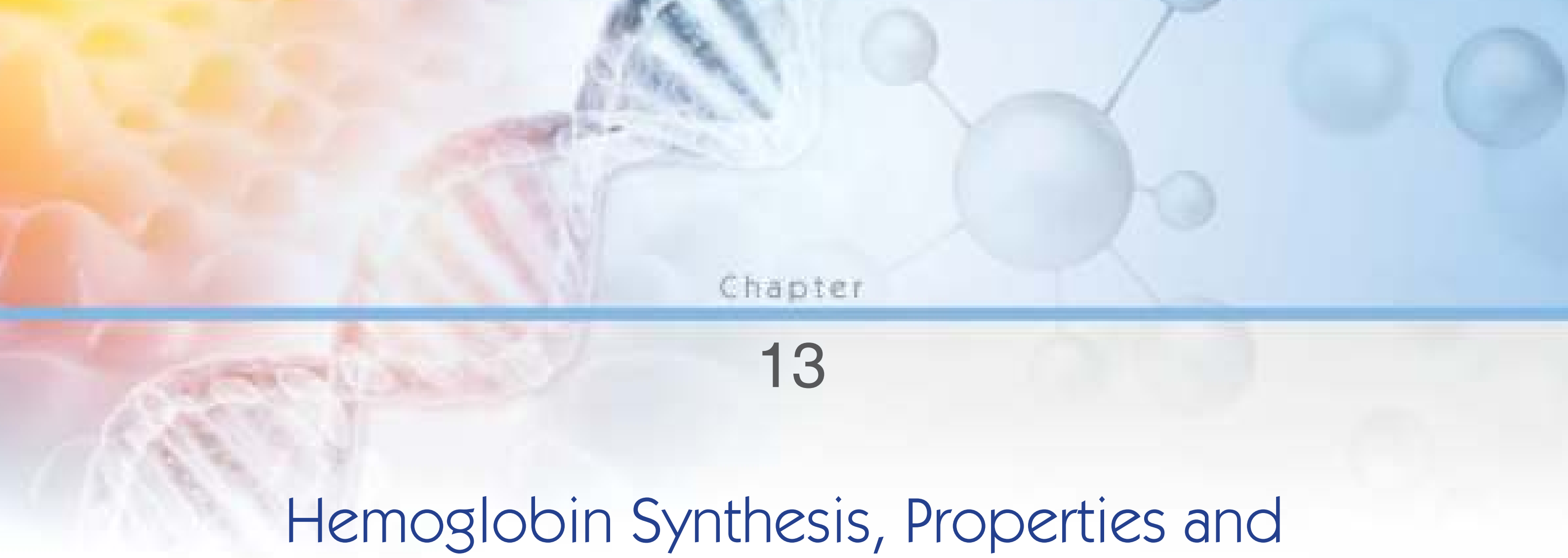
A. Glucuronidation
B. Sulfonation
C. Acetylation
D. Methylation

Q10. Substances such as phenol, arylamines, etc. are removed from the body by

A. Glucuronidation
B. Sulfonation
C. Acetylation
D. Methylation

Answers

1. D **2.** D **3.** B **4.** C **5.** B **6.** A **7.** B **8.** A **9.** C **10.** B



Chapter

13

Hemoglobin Synthesis, Properties and Related Clinical Conditions

SPQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

Hemoglobin is a conjugated protein. It is an iron-containing metalloprotein present in the red blood cells (Fig. 13.1). It gives red color to blood. Hemoglobin in the blood carries oxygen from the lungs to the rest of the body tissues, where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism and collects the resultant carbon dioxide to bring it back to the respiratory organs to be expired from the lungs. When fully saturated each gram of hemoglobin holds approximately 1.34 ml of oxygen. It increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in the blood. The hemoglobin molecule can bind up to four oxygen molecules. Hemoglobin is involved in the transport of other gases also. It carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins (Fig. 13.1). Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure forms from its four subunits in roughly a tetrahedral arrangement.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of the histidine residue below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinated covalent bond. Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions but is bound to the protein chains of the structure.

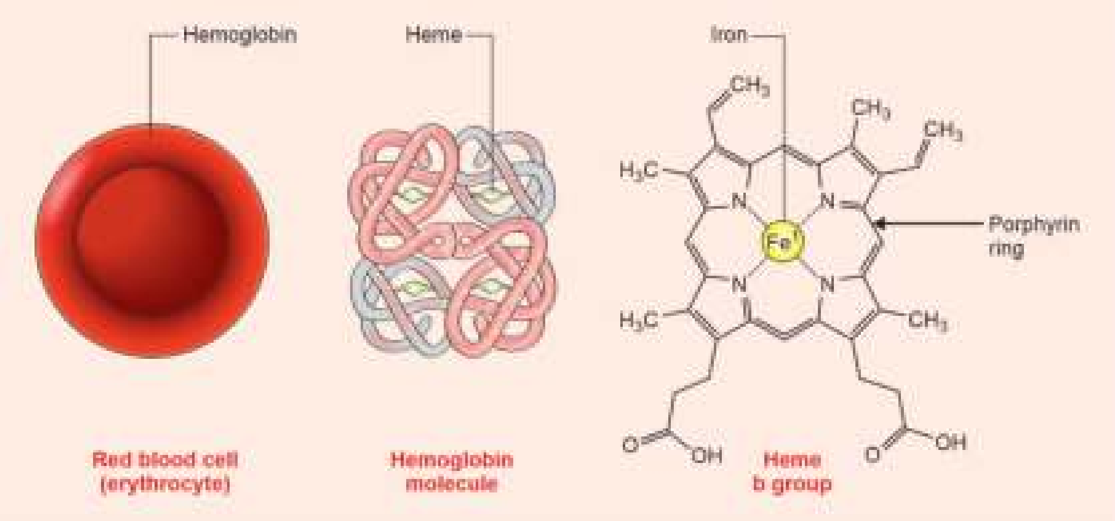


Fig. 13.1: Hemoglobin and porphyrin ring

Oxidation converts hem into hematin, which when linked with globin, gives methemoglobin in which the iron is in the ferric form. Other substances derived from it are oxyhemoglobin, carboxyhemoglobin, and sulfhemoglobin. When the iron is removed, porphyrins are obtained with an intact ring of four pyrrole groups.

The iron in the hemoglobin is in the ferrous state (Fe^{++}). The presence of an enzyme, methemoglobin reductase keeps the iron in the ferrous state. After combining with oxygen (oxy-hemoglobin form) also the iron remains in the ferrous state. The combination of oxygen and hemoglobin is loose and reversible. In some diseased states, however, the iron of hemoglobin gets oxidized and converted into a ferric (Fe^{+++}) state. This condition is called methemoglobinemia and oxygen cannot release itself from methemoglobin easily in the tissues.

SYNTHESIS OF HEMOGLOBIN (Fig. 13.2)

Competency achievement: The student should be able to:

BI6.11: Describe the functions of heme in the body and describe the processes involved in its metabolism and describe porphyrin metabolism

LAQ: Write a note on hemoglobin synthesis.

Ans: The heme part of hemoglobin is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells. The protein globin is synthesized by ribosomes in the cytosol. Production of hemoglobin continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. The nucleus then disappears in mature red blood cells; so that a larger surface area is available for the red cell to carry adequate oxygen for the cells.

The various steps involved in heme synthesis are as follows:

1. The first step in heme synthesis takes place in the mitochondrion. The two starting compounds required are succinyl-CoA (derived from the citric acid cycle) and glycine. Pyridoxal phosphate is necessary to "activate" glycine.
2. The product formed by the condensation reaction between succinyl-CoA and glycine is α-amino-β-ketoadipic acid, which is rapidly decarboxylated to form α-aminolevulinic acid (ALA). This reaction is catalyzed by ALA synthase. In this reaction, pyridoxal phosphate acts as a cofactor.

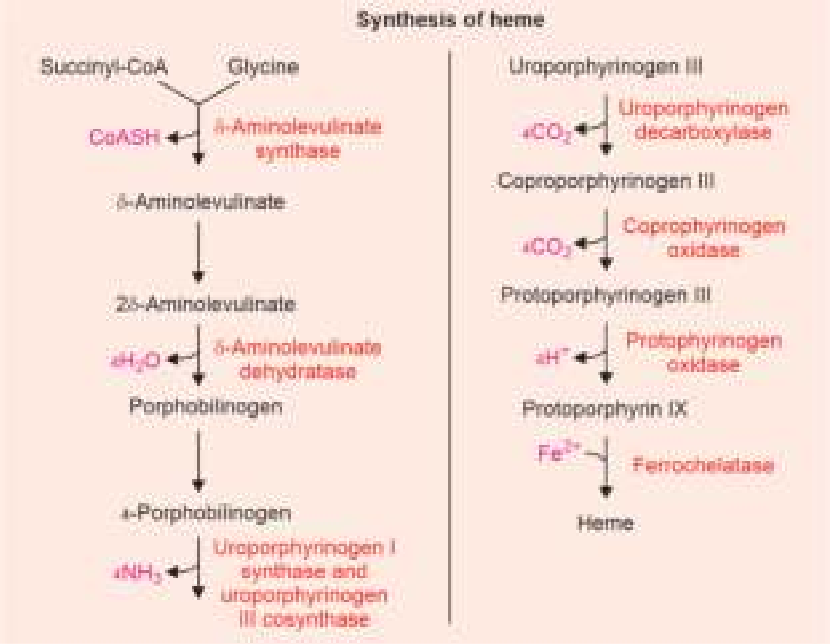

Synthesis of heme
Succinyl-CoA
Glycine
CoASH
δ-Aminolevulinate synthase
δ-Aminolevulinate
2δ-Aminolevulinate
4H₂O
δ-Aminolevulinate dehydratase
Porphobilinogen
4-Porphobilinogen
4NH₃
Uroporphyrinogen I synthase and uroporphyrinogen III cosynthase
Uroporphyrinogen III
4CO₂
Uroporphyrinogen decarboxylase
Coproporphyrinogen III
4CO₂
Coprophyrinogen oxidase
Protoporphyrinogen III
4H⁺
Protophyrinogen oxidase
Protoporphyrin IX
Fe²⁺
Ferrochelatase
Heme

Fig. 13.2: Synthesis of hemoglobin

3. In the cytosol, two ALA molecules are condensed by the enzyme ALA dehydratase to form one molecule of porphobilinogen (PBG) and one molecule of water.
4. Four porphobilinogen molecules get condensed by the enzyme uroporphyrinogen I synthase to form a linear tetrapyrrole, hydroxymethylbilane (HMB). HMB cyclizes spontaneously to form uroporphyrinogen I and III.
5. Uroporphyrinogen I is the precursor of other porphyrins but plays no further part in heme synthesis. Uroporphyrinogen III is the precursor of the porphyrin III series. It is converted to coproporphyrinogen III (a reaction catalyzed by uroporphyrinogen decarboxylase).
6. Coproporphyrinogen III enters the mitochondria, where it is converted to protoporphyrinogen IX and then to protoporphyrin IX.
7. Conversion of coproporphyrinogen III to protoporphyrinogen takes place by the action of the enzyme coproporphyrinogen oxidase.
8. The oxidation of protoporphyrinogen IX to protoporphyrin IX is catalyzed by protoporphyrinogen oxidase.
9. Heme formation takes place by the incorporation of ferrous ions into protoporphyrin IX in a reaction catalyzed by the enzyme heme synthase. Heme synthesis occurs mainly (85%) in erythroid precursor cells in the bone marrow and the majority of the remainder in the hepatocytes.
10. Two distinct globin chains (each with its heme molecule) combine to form hemoglobin. One of the chains is designated alpha. The pairing of one alpha chain and one non-alpha chain produces a hemoglobin dimer (two chains). The hemoglobin dimer does not efficiently deliver oxygen. Two dimers combine to form a hemoglobin tetramer, which is the functional form of hemoglobin.
11. Each molecule of heme combines with one molecule of globin. All hemoglobins

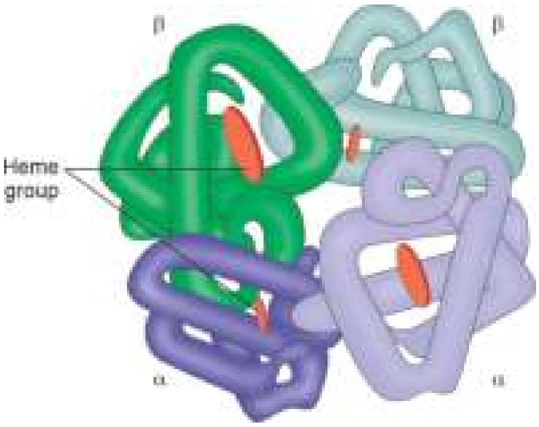


Fig.13.3: Quaternary structure of hemoglobin

contain four pairs of heme + globin with a total molecular weight of about 68,000 (Fig. 13.3).

SAQ: Enumerate the factors required for the synthesis of hemoglobin.

Ans: The following factors are required for hemoglobin synthesis:

1. Amino acids are required for the synthesis of globin.
2. The raw building materials for heme, such as glycine, succinic acid, etc. are available from the metabolic intermediaries.
3. Iron should be supplied in the food.
4. Traces of copper and cobalt are supplied in the food.

Competency achievement: The student should be able to:

BI6.12: Describe the major types of hemoglobin and its derivatives found in the body and their physiological/pathological relevance

Hemoglobin Structure

BAQ: Write a note on hemoglobin structure.

Ans: Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins (Fig. 13.3). Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attraction within the molecule and folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure forms from its four subunits in roughly a tetrahedral arrangement (Fig. 13.2).

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges), with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein through the imidazole ring of the histidine residue below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinated covalent bond.

LAQ: Describe the major types of hemoglobin, its derivatives found in the body, and their physiological and pathological relevance.

Ans: The normally occurring hemoglobins are: (1) A (about 96%), (2) A2: A minor component of A (about 3%), and (3) Fetal hemoglobin (F) (up to 1%). Each molecule of hemoglobin has one globin unit with four hem units.

The globin of each hemoglobin molecule consists of a tetramer with two polypeptide chains of one kind and two of another type.

In normal adult 'A', these chains are called α and β chains; in A2, α and δ chains, and F, α and γ chains.

Each chain is composed of a sequence of about 150 amino acid units. The substitution of any one of these amino acids by another results in the formation of an abnormal chain and of an abnormal hemoglobin. Amounts in excess over A2, are associated with the condition β-thalassemia. In this condition, there is suppression of beta chain formation. In α-thalassemia, there is a corresponding suppression of α chains. This accordingly leads to failure of the formation of A, A_2, and F, and hemoglobin such as those with four β or four γ chains appear.

The more commonly occurring abnormal hemoglobin is: (1) Hemoglobin 'S', which is present in red blood cells in sickle cell anemia, (2) Hemoglobin C and D are found in some black populations, and (3) Hemoglobin E in many Thais.

Hemoglobin in the blood can undergo auto-oxidation. Similarly, many drugs such as sulfanilamide, phenacetin, etc. and toxins such as phenylhydrazine, nitrites, etc. oxidize heme in hemoglobin. Oxidation converts heme into hematin. When hematin links with globin, gives methemoglobin, in which the iron is in the ferric form. Other substances derived from hemoglobin are oxyhemoglobin (combination with oxygen) carboxyhemoglobin, (combination with carbon monoxide), and sulfhemoglobin. (formed by the action of sulfur-containing drugs).

The iron in the hemoglobin must remain in the ferrous (Fe^{++}) state to bind with oxygen. Temporary oxidation of Fe^{++} to Fe^{+++} leads to the formation of methemoglobin. In the methemoglobin form, the ability of hemoglobin to release oxygen to the cells is reduced significantly. Normally, methemoglobin levels are < 1%. The enzyme NADH methemoglobin reductase acts on methemoglobin to form hemoglobin in the reduced state (Fe^{++}). Elevated levels of methemoglobin in the blood are caused due to certain congenital deficiencies of the enzyme NADH methemoglobin reductase. In this clinical condition, oxygen-carrying ferrous ion (Fe^{2+}) of the heme group of the hemoglobin molecule is oxidized to the ferric state (Fe^{3+}). This converts hemoglobin to methemoglobin, resulting in a reduced ability to release oxygen to tissues, and thereby causing hypoxia. This can give the blood a bluish or chocolate-brown color.

SAQ: In which clinical condition does methemoglobin in the blood rises? Why significant increase in methemoglobin gives rise to hypoxia?

Ans: Elevated levels of methemoglobin in the blood are caused due to certain congenital deficiencies of the enzyme NADH methemoglobin reductase. In this clinical condition, oxygen-carrying ferrous ion (Fe^{2+}) of the heme group of the hemoglobin molecule is oxidized to the ferric state (Fe^{3+}). This converts hemoglobin to methemoglobin, resulting in a reduced ability to release oxygen to tissues, and thereby causing hypoxia

SAQ: Write four names of hemoglobin derivatives.

Ans: Methemoglobin, sulfhemoglobin, oxyhemoglobin, and carboxyhemoglobin.

SAQ: Write a short note on the oxygen-binding capacity of hemoglobin.

Ans: Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin which increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in the blood. The hemoglobin molecule can bind up to four oxygen molecules. Hemoglobin is involved in the transport of other gases also. It carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

PORPHYRINS AND DISORDERS OF HEME SYNTHESIS-PORPHYRIAS

LAQ: Write a note on porphyrin metabolism, porphyrias, and diagnostic test to detect porphyria.

Ans: When the iron is removed, porphyrins are obtained with the intact ring of four pyrrole groups. Porphyrins are biologically important heterocyclic compounds of a characteristic chemical structure that includes four pyrrole groups (five-membered organic rings, each containing a nitrogen atom) linked by additional carbon atoms to form a large flat ring. As biological pigments, they are responsible for many of the colors in living organisms. They often occur combined with metal ions and various substituents as coordination complexes. Various examples of porphyrins are uroporphyrin, coproporphyrin, and protoporphyrin.

Porphyrins are synthesized as follows (Fig. 13.2):

1. In the cytosol, two aminolevulinic acid (ALA) molecules are condensed by the enzyme ALA dehydratase to form one molecule of porphobilinogen (PBG) and one molecule of water.
2. Four porphobilinogen molecules get condensed by the enzyme uroporphyrinogen I synthase to form a linear tetrapyrrole, hydroxymethylbilane (HMB). HMB cyclizes spontaneously to form uroporphyrinogen I and III.
3. Uroporphyrinogen I is the precursor of other porphyrins but plays no further part in heme synthesis. Uroporphyrinogen III is the precursor of the porphyrin III series. It is converted to coproporphyrinogen III. This reaction is catalyzed by the enzyme, uroporphyrinogen decarboxylase.
4. Coproporphyrinogen III enters the mitochondria, where it is converted to protoporphyrinogen IX and then to protoporphyrin IX.
5. Conversion of coproporphyrinogen III to protoporphyrinogen takes place by the action of the enzyme coproporphyrinogen oxidase.
6. The oxidation of protoporphyrinogen IX to protoporphyrin IX is catalyzed by protoporphyrinogen oxidase.

Porphyrias

Porphyrias are mainly a group of inborn errors of metabolism associated with the biosynthesis of heme.

Most of the porphyrias are inherited as autosomal dominant traits and caused mainly due to deficiency of enzymes such as ALA synthase, ALA dehydrogenase, uroporphyrinogen I synthase, uroporphyrinogen III, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase, protoporphyrinogen oxidase, and heme synthase, etc. required for the synthesis of heme.

The porphyrias are characterized by increased production and excretion of porphyrins and/or their precursors, i.e. aminolevulinic acid (ALA) and porphobilinogen (PBG). The main problem in these enzyme deficiencies is the accumulation of porphyrins (the heme precursors), which are toxic to tissue in high concentrations.

The following are various types of porphyrias: Acquired porphyrias, acute porphyrias, and cutaneous porphyrias.

Acquired porphyrias may cause severe liver disease and in some cases of mercury- or arsenic poisoning. This type of porphyria could be treated when the primary cause is taken into consideration for the treatment.

Acute porphyrias are a group of rare disorders characterized by an enzymatic defect in the heme biosynthesis.

Acute (or hepatic) porphyrias primarily affect the nervous system, resulting in abdominal pain, vomiting, acute neuropathy, muscle weakness, seizures, and mental disturbances, including hallucinations, depression, anxiety, and paranoia. Cardiac arrhythmias and tachycardia may develop as the autonomic nervous system is affected. Pain can be severe and chronic. Constipation is frequently present, as the nervous system of the gut is affected, and diarrhea can also occur.

Cutaneous Porphyrias

The cutaneous, or erythropoietic porphyrias primarily affect the skin, causing photosensitivity (photodermatitis), blisters, necrosis of the skin and gums, itching, and swelling, and increased hair growth on areas such as the forehead. Abdominal pain may not be observed, distinguishing it from other porphyrias.

Diagnostic test

1. Porphyria is diagnosed using spectroscopy methods and biochemical analysis of blood, urine, and stool for the determination of porphyrins.
2. The genetic carriers of the more common, dominantly inherited acute hepatic porphyrias can be detected by DNA tests.

SAQ: Write the principle of pulse oximeter and use in COVID-19.

Ans: Oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm, its absorption is slightly higher. This difference is used for the measurement of the amount of oxygen in a patient's blood by an instrument called a pulse oximeter. This difference also accounts for the presentation of cyanosis, the blue-to-purplish color that tissues develop during hypoxia. A pulse oximeter is extremely useful in the determination of low oxygen saturation in respiratory distress syndrome in COVID-19 and also in severe lung diseases.

SAQ: Enumerate symptoms of acute porphyris.

Ans: Acute porphyrias primarily affect the nervous system, resulting in abdominal pain, vomiting, acute neuropathy, muscle weakness, seizures, and mental disturbances, including hallucinations, depression, anxiety, and paranoia. Cardiac arrhythmias and tachycardia may develop as the autonomic nervous system is affected. Patients may suffer from severe pain, constipation, or diarrhea.

PROPERTIES AND FUNCTIONS OF HEMOGLOBIN

SAQ: Write three important functions of hemoglobin.

Ans:

1. When fully saturated, each gram of hemoglobin holds approximately 1.34 ml of oxygen. Hemoglobin in the blood carries oxygen from the lungs to the rest of the body tissues, where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism
2. Hemoglobin collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism. Hemoglobin also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group. Nitric acid plays an important role in dilating arteries to provide more oxygen supply to cells in hypoxia.
3. Hemoglobin plays an important role as a buffer molecule in the maintenance of an acid–base balance in the body. In oxyhemoglobin form, it donates protons, and in the deoxygenated form, it accepts protons.

Binding Ability of Hemoglobin (Fig. 13.3)

LAQ: Write a note on the binding ability of hemoglobin.

Ans: Oxyhemoglobin is formed during physiological respiration when oxygen binds to the heme component of hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the bloodstream to the cells, where it is utilized in glycolysis and the production of ATP by the process of oxidative phosphorylation.

Hemoglobin exists in two forms, a taut form (T) and a relaxed form (R). Various factors such as low pH, high CO_2, and high 2,3-BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. Low CO_2 and low 2,3-BPG at the level of the lung capillaries favor the relaxed form of hemoglobin, which can better bind oxygen.

Hemoglobin molecule also binds to competitive inhibitors such as carbon monoxide (CO) and allosteric ligands such as nitric oxide (NO). Nitric oxide binds to specific thiol groups in the globin protein to form a S-nitrosothiol which dissociates into free nitric oxide and thiol again as the hemoglobin releases oxygen from its heme site. This nitric oxide transport to peripheral tissues facilitates oxygen transport in tissues by releasing vasodilatory nitric oxide to tissues in which oxygen levels are low.

The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygen in a way favorable for binding. This positive, cooperative binding is achieved through steric conformational changes in the hemoglobin protein complex. When one subunit protein in hemoglobin becomes oxygenated, a conformational or structural change in the whole complex is initiated, causing the other subunits to gain an increased affinity for oxygen. Hence, the oxygen binding curve of hemoglobin is sigmoidal, or S-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding (Fig. 13.4).

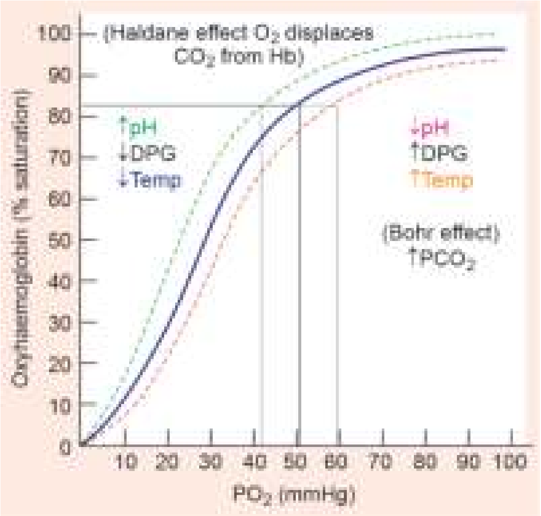


Fig. 13.4: Sigmoid oxygen binding curve of hemoglobin

The oxygen-binding capacity of hemoglobin is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin. Carbon monoxide has almost 200 times stronger affinity for hemoglobin than that oxygen for hemoglobin. Carbon monoxide hemoglobin is formed in the blood, which prevents the formation of oxyhemoglobin, leading to hypoxia.

When hemoglobin combines with CO, it forms a bright red compound called carboxyhemoglobin, which may cause the skin of CO poisoning victims to appear pink in death instead of white or blue.

When inspired air contains CO levels as low as 0.02%, headache and nausea occur. If the CO concentration is increased to 0.1%, unconsciousness will follow. In heavy smokers, up to 20% of the oxygen-active sites can be blocked by CO.

Hemoglobin also has a competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide (S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding, causing severe toxicity.

SAQ: What is the significance of a taut and relaxed form of hemoglobin?

Ans: Hemoglobin exists in two forms, a taut form (T) and a relaxed form (R). Various factors such as low pH, high CO_2, and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. Low CO_2 and low 2,3 BPG at the level of the lung capillaries favor the relaxed form of hemoglobin, which can better bind oxygen.

SAQ: Write a note on carbon monoxide poisoning, related symptoms and first line of treatment.

Ans: The binding of oxygen to hemoglobin is affected by carbon monoxide (CO), which is involved in tobacco smoking, fuel-burning appliances in the house, released from car exhaust, and incomplete combustion in furnaces.

The oxygen-binding capacity of hemoglobin is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin. Carbon monoxide has almost 200 times stronger affinity for hemoglobin than that oxygen for hemoglobin. Carbon monoxide hemoglobin is formed in the blood, which prevents the formation of oxyhemoglobin, leading to hypoxia. In heavy smokers, up to 20% of the oxygen-active sites can be blocked by CO.

CO competes with oxygen at the heme binding site. When hemoglobin combines with CO, it forms a bright red compound called carboxyhemoglobin, which may cause the skin of CO poisoning victims to appear pink in death instead of white or blue.

When inspired air contains CO levels as low as 0.02%, headache and nausea occur. If the CO concentration is increased to 0.1%, unconsciousness will follow. If the patient is not treated in time, the patient may slip into a coma and may die.

Symptoms: The following are the symptoms of severe CO poisoning: Shortness of breath, headache, ataxia, nausea, altered mental status, chest pain, tachycardia, irritability, and loss of consciousness in severe poisoning.

First-line of treatment: Determination of blood oxygen saturation by pulse oximeter and accordingly administration of oxygen until the patient is symptom-free. It is necessary to perform serial neurologic examinations to assess the improvement in the clinical conditions of the patient.

SAQ: Name five poisonous gases that cause severe toxicity by binding to oxygen.

Ans: Hemoglobin has a competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide (S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding, causing severe toxicity.

Haldane Effect

BAQ: What is Haldane effect?

Ans: Carbon dioxide formed in the metabolic reaction occupies a different binding site on the hemoglobin. Carbon dioxide dissolves more readily in deoxygenated blood, facilitating its removal from the body after the oxygen has been released to tissues undergoing metabolism. This increased affinity for carbon dioxide by the hemoglobin in venous blood is known as the Haldane effect.

By the action of the enzyme carbonic anhydrase, carbon dioxide reacts with water to form carbonic acid, which dissociates into bicarbonate and protons:

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3 \longrightarrow HCO_3^- + H^+$$

SAQ: What are allosteric effectors of hemoglobin?

Ans: The ions that cause a change in the allosteric status are called “allosteric effectors”. H^+ ions, 2,3 BPG, CO_2, and Cl^- are called "allosteric effectors". All these ions affect the allosteric status of hemoglobin. Bicarbonate, formed in the chemical reaction, moves out of red blood cells, and an equal amount of chloride ions enter red blood cells and bind deoxyhemoglobin.

Bohr Effect

BAQ: What is the Bohr effect?

Ans: Hemoglobin in the blood acts as a buffer. In oxyhemoglobin form, it donates protons, and in the deoxygenated form, it accepts protons. Hemoglobin can bind protons and carbon dioxide, which causes a conformational change in the protein and facilitates the release of oxygen. Protons bind at various places on the protein, while carbon dioxide binds at the α-amino group. Carbon dioxide binds to hemoglobin and forms carbaminohemoglobin. This decrease in hemoglobin’s affinity for oxygen by the binding of carbon dioxide and protons is known as the Bohr effect (shifts the O_2^- saturation curve to the right) (Fig. 13.4).

The Bohr effect is very important, since, during increased tissue activities, more CO_2 is produced. These additional CO_2

concentrations are sensed by the hemoglobin in red blood cells and cause them to release more O_2 in those places where it is most required. This is an important self-regulating mechanism for efficient oxygen transport of cells.

SAQ: What is the root effect?

Ans: A reduction in the total binding capacity of hemoglobin to oxygen due to increased proton and carbon dioxide concentration by decreasing blood pH is called the root effect.

BAQ: Write a note on the role of 2,3-Bisphosphoglyceric acid (2,3-BPG).

Ans: 2,3-Bisphosphoglyceric acid is a three-carbon isomer of the glycolytic intermediate 1,3-bisphosphoglyceric acid (1,3-BPG). 2,3-BPG is present in human red blood cells at approximately 5 mmol/L. It binds with greater affinity to deoxygenated hemoglobin (e.g. when the red cell is near respiring tissue) than it does to oxygenated hemoglobin (e.g. in the lungs) due to spatial changes. It fits in the deoxygenated hemoglobin configuration but not as well in the oxygenated hemoglobin. It interacts with deoxygenated hemoglobin beta subunits by decreasing their affinity for oxygen. This means it allosterically promotes the release of the oxygen molecules bound to the hemoglobin, thus enhancing the ability of RBCs to release oxygen near tissues that need it the most. 2,3-BPG is thus an allosteric effector, which promotes the release of oxygen in tissues and acceptance of oxygen in the lungs when oxygen is available through inspired air.

SAQ: Why 2,3-BPG is increased in persons living at high altitudes?

Ans: In people acclimated to high altitudes, the concentration of 2,3-Bisphosphoglycerate (2,3-BPG) in the blood is increased, which allows these individuals to deliver a larger amount of oxygen to tissues under conditions of lower oxygen tension.

SAQ: Why in severe anemia, does 2,3-BPG concentration increase in blood?

Ans: In severe anemia, 2,3-BPG concentration increases to increase the supply of oxygen to tissues.

SAQ: Why inosine is added in blood collected at blood banks?

Ans: The addition of inosine prevents the decrease of 2,3-BPG in stored blood meant for blood transfusion. 2,3-BPG facilitates the transfer of a larger amount of oxygen to tissues in the acceptor patient.

SAQ: What is the function of fetal hemoglobin?

Ans: Fetal hemoglobin (HbF, $\alpha_2\gamma_2$), is found in the developing fetus and binds oxygen with greater affinity than adult hemoglobin. This means the oxygen binding curve for fetal hemoglobin is left-shifted (i.e. a higher percentage of hemoglobin has oxygen bound to it at lower oxygen tension) in comparison to that of adult hemoglobin. As a result, fetal blood in the placenta can take oxygen from maternal blood. Fetal hemoglobin (HbF) exhibits a low affinity for 2,3-Bisphosphoglyceric acid (BPG), resulting in a higher binding affinity for oxygen.

BAQ: Write a note on the Rapoport-Luebering cycle (shunt).

Ans: The Rapoport-Luebering cycle (Fig. 13.5) is a metabolic pathway in mature erythrocytes that facilitates the formation of 2,3-bisphosphoglycerate (2,3-BPG); which is required to regulate oxygen release from hemoglobin and delivery to tissues, during systemic circulation of blood. When 2,3-BPG binds hemoglobin, it decreases the affinity of hemoglobin for oxygen; which is then released and cells can use oxygen for cellular respiration.

About 15–20% of the glucose that gets converted to lactate in erythrocytes through Embden-Meyerhof pathways goes through the Rapoport-Luebering cycle to produce 2,3-BPG synthesis. The following are the main steps of the Rapoport-Luebering cycle:

Bisphosphoglycerate mutase acts on 1,3-Bisphosphoglycerate formed in glycolysis to form 2,3-Bisphosphoglycerate.

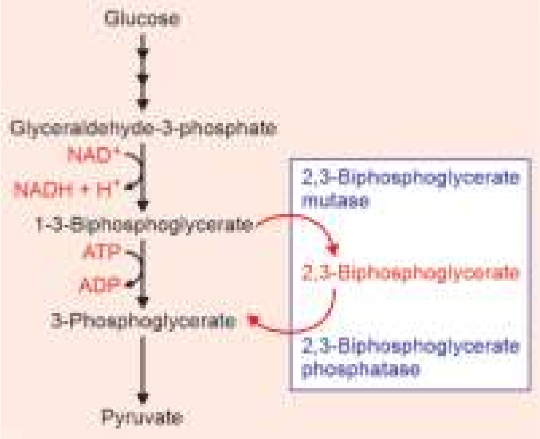


Fig. 13.5: Rapoport-Luebering cycle

2,3-Bisphosphoglycerate combines with hemoglobin and oxygen is released to the cells.

2,3-Bisphosphoglycerate phosphatase acts on 2,3-Bisphosphoglycerate to form 3-phosphoglycerate, which is ultimately converted to pyruvate.

Total 2 ATP molecules are generated and two ATP molecules are consumed in the Rapoport-Luebering cycle.

Competency achievement: The student should be able to:

BI7.6: Describe the antioxidant defense systems in the body

Red Cell Enzymes

BAQ: Write a note on red blood cell enzymes.

Ans: Red cells contain the following two classes of enzymes:

1. Remodeling enzymes: These are responsible for the degradation and disposal of reticulocyte organelles, such as mitochondria and ribosomes. *Example:* Pyrimidine 5′–nucleotidase. This enzyme facilitates the removal of residual ribonucleotides from maturing reticulocytes.
2. Housekeeping enzymes: These are responsible for maintaining red cell integrity and function. These enzymes are more frequently responsible for hemolytic disorders when they undergo mutation. The normal red cell is equipped with several enzymes that protect against oxidative damage, e.g. catalase, glutathione peroxidase and superoxide dismutase.

SAQ: What is the importance of the Embden-Meyerhof (E-M) pathway, in the maintenance of red blood cells?

Ans: The Embden-Meyerhof (E-M) pathway regenerates ATP molecules, which are used to energize the sodium/potassium and calcium pumps that maintain the normal internal ionic environment of red cells. The ATP molecules are also used to phosphorylate certain membrane components and for the synthesis of glutathione).

Another role of the E-M pathway is to reduce NAD to NADH. NADH is required to reduce methemoglobin (Fe^{+++}) to the functional Fe^{++} form.

BAQ: What is the role of Glutathione and the pentose phosphate shunt (PPS) in the maintenance of red blood cells?

Ans: Glutathione is a thiol tripeptide (γ-glutamyl-cysteinyl-glycine). It acts as a reducing agent and is found in most cells. During the metabolic reactions, glutathione reductase acts on the oxidized form of glutathione (GSSG) in the presence of NADPH, and it is converted to reduced GSH. Reduced GSH acts with the catalytic action of the enzyme glutathione peroxidase to destroy toxic hydrogen peroxide, which forms in the metabolic reactions. In this process, GSH is converted back to GSSG (oxidized form), and hydrogen peroxide is converted to water. GSH is also required to maintain the integrity of sulfhydryl groups and various enzymes present within the membrane of a red blood cell.

The pentose phosphate shunt (PPS) maintains the appropriate levels of NADPH required for the reduction of oxidized glutathione (GSSG). For the formation of

NADPH, the initial action of Glucose-6-PD is necessary for PPS pathways, which produce NADPH molecules.

BAQ: What is the function of the Glucose-6-phosphate dehydrogenase (G6-PD) enzyme in red blood cell membranes, and clinical significance of congenital deficiency of G6-PD?

Ans: G6-PD catalyzes the first, pace-making reaction of the pentose phosphate cycle (PPC) which produces NADPH. NADPH maintains glutathione and thiol groups of proteins and enzymes in the reduced state which is essential for protection against oxidative stress. In oxidative stress, hydrogen peroxide is produced, which is toxic to the cells. However, by the action of glutathione and glutathione peroxide, hydrogen peroxide is eliminated as shown in the following reaction:

$$\text{Glucose 6-P} + \text{NADP} \xrightarrow{\text{G6-PD}} \text{Phosphogluconic acid} + \text{NADPH} + \text{H}^+$$

$$\text{NADPH} + \text{H}^+ + \text{GSSG} \xrightarrow{\text{Gultathione reductase}} \text{NADP}^+ + 2\text{GSH}$$

$$\text{H}_2\text{O}_2 + 2\text{GSH} \xrightarrow{\text{Glutathione peroxidase}} \text{GSSG} + 2\text{H}_2\text{O}$$

In the congenital deficiency of G6-PD, a large amount of hydrogen peroxide produced, particularly by the action of antimalarial drugs, is not destroyed. Excessive formation of hydrogen peroxide leads to hemolysis of red blood cells, leading to hemolytic anemia.

RED CELL DESTRUCTION: PATHOLOGIC AND PHYSIOLOGIC

LAQ: Write a note on various factors responsible for red cell destruction.

Ans: The various factors responsible for red blood cell destruction are as follows:

1. Environmental factors: A variety of injurious environmental factors, such as a diet deficient in iron, other micronutrients, B-complex vitamins, radiations, and high temperature, may lead to premature red cell destruction.
2. Mutations: By mutation that leads to an intrinsic structural alteration of the red cell may predispose to a congenital hemolytic process. Mutations in the alpha or beta chains of hemoglobin may lead to decreased solubility of the molecule or its increased susceptibility to oxidation and denaturation.
3. Excessive mechanical stress originating in malfunctioning prosthetic heart valves or from intravascular deposition of fibrin clots.
4. Red cell membrane destabilization due to excessive heating (as in burns).
5. Many chemical compounds can induce red cell injury and destruction. Various examples are copper, arsenic, chloramine, and lipolytic toxins produced by various microorganisms, plants, and animals.
6. Phosphatidyl serine abnormality in the red cell membrane usually results in a decreased ability of the red cell membrane or hemoglobin to withstand oxidative stress.
7. Immunologic factors: Hemolysis can be caused by immunologic processes that induce the binding of specific antibodies or complement components to red cell membranes. Immune hemolytic disorders may be triggered by drugs as well as autoimmune antibodies.
8. Enzymopathies (congenital deficiency of enzymes), involving the E-M pathway may lead to ATP deficiency with impaired red cell formation.

IRON METABOLISM

Competency achievement: The student should be able to:

PE13.1: Describe the RDA, dietary sources of iron, and their role in health and disease

Refer to Chapter 8, page 191.

Competency achievement: The student should be able to:

PE13.3: Describe the causes, diagnosis, and management of iron deficiency

Competency achievement: The student should be able to:

PE13.4: Describe the hemogram and iron panel

IMPORTANCE OF HEMOGRAM AND IRON PANEL TESTS

Refer to Chapter 8, page: 195

DISORDERS OF HB STRUCTURE AND SYNTHESIS

BAQ: Write a note on the hereditary disorders of hemoglobin.

Ans: The hereditary disorders of Hb may be classified into two broad groups hemoglobinopathies and thalassemias.

1. Hemoglobinopathies are characterized by the production of structurally defective hemoglobin due to abnormalities in the formation of the globin moiety of the molecule.

   Examples: Sickle cell trait and sickle cell disease.
2. The thalassemias are characterized by a reduced rate of production of normal hemoglobin due to the absent or decreased synthesis of one or more types of globin polypeptide chains.

Abnormal hemoglobins are inherited as autosomal co-dominants. Thus, subjects who inherit one normal and one abnormal gene are heterozygotes, and those who have two identical abnormal genes are homozygotes. Double heterozygotes are subjects who have inherited two different abnormal genes. The homozygous state is usually referred to as the 'disease'. For example, the homozygous state for HbC is "Hb-disease", and the heterozygous state is the 'trait'. This rule has some exceptions. Beta chains take part in the formation of HbA only, and thus β-chain variants are all variants of HbA.

Carboxyhemoglobin

SAQ: Why a person gets seriously affected by carbon monoxide poisoning?

Ans: When carbon monoxide (CO) binds to hemoglobin, carboxyhemoglobin forms, and the affinity of CO to Hb is 200 times more than that of oxygen. After the formation of carboxyhemoglobin, it becomes unsuitable for oxygen transport. The carboxyhemoglobin level in normal persons is about 0.16%. Clinical symptoms manifest when carboxyhemoglobin levels exceed 20%. The common clinical symptoms of carbon monoxide poisoning are shortness of breath, severe headache, confusion, dizziness, convulsions, fainting, and coma. Carbon monoxide poisoning may be life-threatening if not treated in time.

Classification of Hemoglobin Variants

BAQ: Write a note on the classification of hemoglobin variants.

Ans: The hemoglobin variants mostly may be either the alpha chain variant or the beta chain variant. Although rarely, gamma and delta chain variants have also been found.

A large number of Hb variants may be classified into the following 5 major types. These are based on their manifestations:

A. Sickle syndromes
   1. Sickle-cell trait (AS)
   2. Sickle-cell disease with SS, SC, SD types, and S beta-thalassemia.

B. The unstable hemoglobins (congenital Heinz body anemia).

C. Hemoglobins with abnormal oxygen affinity.
   1. High affinity (familial polycythemia)
   2. Low affinity (familial cyanosis).

D. Structural variations
   1. Alpha thalassemia
   2. Beta thalassemia

E. Various other types, such as HbP, Q, N, J, etc. do not produce clinical symptoms.

Sickle Cell Hemoglobin (HbS)

BAQ: Write a note on sickle cell hemoglobin (HbS)

Ans: The abnormality in the structure of HbS is due to the substitution of normal glutamic acid by an abnormal valine in the 6th position in the beta chain. This leads to the polymerization of Hb molecules due to partial or full deoxygenation. As a result, intracellular fibers are formed, which distort the red blood cell into a sickle shape (Fig. 13.6). Due to polymerization and sickling, the viscosity of blood increases, and it slows down blood circulation.

In the heterozygous (AS) condition, 50% Hb in red blood cells is normal; hence the sickle cell trait may not produce clinical symptoms. The heterozygous state is found to be very common in Central and West Africa and East-Central India.

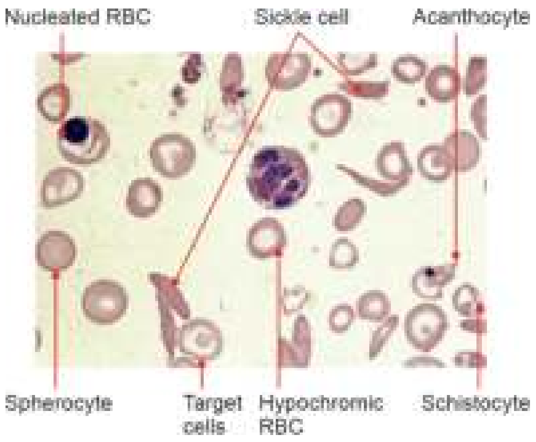


Fig. 13.6: Sickle cells, spherocytes, target cells, nucleated RBC, schistocyte, acanthocyte

STRUCTURAL VARIANTS OF HEMOGLOBIN AND THALASSEMIA SYNDROMES

BAQ: Write a note on thalassemia syndrome.

Ans: Thalassemia syndromes are characterized by genetic defects in the synthesis of one or more of the globin chains that form the hemoglobin tetramer. The different forms of thalassemia arise due to a defect in the mRNA for the affected globin chain. Defects exist at several levels, like operator gene defect, abnormal post-transcriptional processing, lowered stability of the mutant mRNA, loss of start signals for translation of mRNA, and nonsense mutations leading to premature chain termination.

The clinical syndromes associated with thalassemia arise from the combined consequences of inadequate hemoglobin production and unbalanced accumulation of globin chains. The former causes anemia with hypochromia and microcytosis, and the latter leads to ineffective erythropoiesis and hemolysis. Clinical manifestations range from completely asymptomatic microcytosis to profound anemia incompatible with life and even death *in utero*.

The α- and β-chains of hemoglobins are synthesized independently under separate genetic control, and, in the normal state, the synthesis of the two chains is balanced. One group of thalassemia affects β-chains and is known as β-thalassemia, and the one which affects α-chains is known as α-thalassemia.

In β-thalassemia the inadequate production of β-chains leads to a reduction in HbA in red blood cells. This leads to an increase in γ- and δ-chains, with an increase in HbF and HbA_2. These γ- and α-chains aggregate and interfere with erythroid cell maturation leading to ineffective erythropoiesis.

In α-thalassemia, the levels of HbA, HbF, and HbA_2 are equally depressed since they all have α-chains. There is usually microcytic hypochromic anemia.

Competency achievement: The student should be able to:

PA16.1: Define and classify hemolytic anemia

THE HEMOLYTIC ANEMIAS

BAQ: Define and classify hemolytic anemia.

Ans: Hemolytic anemias result from an increase in the rate of red cell destruction. The lifespan of the normal red cell is 100–120 days. In hemolytic anemias, the lifespan of red blood cells is decreased by varying degrees.

Anemia, in these cases, develops due to the inability of the bone marrow to compensate for the degree of destruction of red blood cells. Hemolytic anemia may not be seen until the red cell lifespan is less than 30 days.

The hemolytic anemias may be classified into two broad groups:

1. Intracorpuscular or intrinsic abnormality due to a corpuscular defect. These are mainly congenital, and the basic defect may be in any of the following three main components of the cell: The membrane, the hemoglobin molecule, and the enzymes concerned with cell metabolism.

   Examples: Congenital defect in the alpha and beta chains of hemoglobin, leading to alpha- and beta-thalassemia. It may be due to red blood cell membrane defects, as seen in hereditary spherocytosis, elliptocytosis, etc. Due to these defects life of red blood cells decreases significantly, leading to hemolytic anemia.
2. Extracorpuscular or extrinsic abnormality due to an abnormal hemolytic mechanism. These disorders are acquired, and hemolysis may result from either an immune or a non-immune mechanism.

   Examples: Hemolytic anemia due to the destruction of red blood cells due to burns, direct action of drugs and heavy metals like lead, autoantibodies, and microbial infections caused by bacteria (brucella) and parasites (malarial parasite).

LABORATORY DIAGNOSIS OF HEMOLYTIC ANEMIA

BAQ: Enumerate blood tests for the diagnosis of hemolytic anemia.

Ans: The following blood tests are recommended for the diagnosis of hemolytic anemia:

1. Complete hemogram.
2. Microscopic examination of blood film for—spherocyte, schistocyte (red cell fragment), sickle cells, target cells, elliptocytes (oval-shaped RBCs), acanthocytes, (RBCs with sharp projections), stomatocytes (oval shaped central pallor), polychromasia (immature red blood cells), parasites (e.g. malarial parasite), etc (Ref: Fig. 13.6, Fig. 11.6 P.254, Fig. 11.7 P 259, Fig. 11.8. P260).
3. Blood tests for hereditary hemolytic anemias (for red cell membrane abnormality)
   - Osmotic fragility, incubation osmotic fragility, auto hemolysis.
4. Identification of abnormal hemoglobin
   - Hemoglobin electrophoresis.
   - Determination of HbA_2 by HPLC
   - Determination of HbF by alkali denaturation.
   - Quantitation of α and β chains of hemoglobin by HPLC
   - Determination of HbS by sickling test
5. Determination of compensatory erythropoietic hyperplasia
   - Reticulocyte count
   - Nucleated red cell count
6. Biochemical tests to find out the increase in hemolysis of red blood cells
   - Serum bilirubin (total, direct, indirect)
   - Serum LDH and SGOT
   - Urine urobilinogen.

Competency achievement: The student should be able to:

PA16.4: Describe the pathogenesis and clinical features and hematological indices and peripheral blood picture of acquired hemolytic anemia

Competency achievement: The student should be able to:

PA16.2: Describe the pathogenesis and clinical features and hematological indices of hemolytic anemia

Expected Test Results in Hemolytic Anemia

LAQ: Describe the pathogenesis and clinical features and hematological indices of hemolytic anemia.

Ans: Hemolytic anemia is defined as anemia that results from an increase in the rate of red cell destruction. The hemolytic nature of the anemia is determined by evidence of increased hemoglobin breakdown and bone marrow regeneration. Irrespective of their etiology, these features are common to all hemolytic anemias. Hemolytic anemia may not be seen until the red cell lifespan is less than 30 days. Hemolytic anemia can be intravascular or extravascular.

Pathogenesis: Due to excessive hemolysis, the hemoglobin from the destroyed red cells is liberated into the plasma. When the amount of hemoglobin released exceeds the haptoglobin-binding capacity, part of the unbound hemoglobin passes the renal glomerular membrane. It is reabsorbed in the proximal renal tubules. Hemoglobin appears in the urine if the absorptive capacity of the tubules is exceeded.

In the renal tubular cell, the globin is degraded to amino acids, which return to the body stores. The hem is catabolized to bilirubin. Hem iron enters a temporary storage depot in the cell. The presence of iron-laden tubular cells in the urine results in the appearance of urinary hemosiderin.

Part of the circulating unbound hemoglobin is converted to methemoglobin, which dissociates into ferric heme and globin. If the binding capacity of hemopexin is exceeded, the ferric heme is bound by albumin in a 1:1 molar ratio with the formation of methemalbumin. It is the last hem pigment to leave plasma after an episode of intravascular hemolysis. The new part of the methemalbumin molecule is eventually taken up by the parenchymal cells of the liver. The free remains of hemoglobin are taken up by liver cells.

Due to the increased breakdown of red blood cells and metabolism of protoporphyrin, the concentration of indirect bilirubin increases in the blood leading to prehepatic jaundice. Although clinical jaundice is usual in hemolytic anemia, it is only evident when serum total bilirubin exceeds 2.0 mg/dl concentration. Hence, the absence of jaundice does not exclude the diagnosis of hemolytic anemia.

Since red blood cells contain SGOT and LDH, in hemolytic anemia, due to excessive hemolysis of red blood cells, plasma SGOT and LDH values increase above normal levels.

The following are the expected hematological indices of hemolytic anemia:

Hemoglobin and red blood cell count:

- Reduction in hemoglobin is often proportional to a fall in RBC count.
- PCV is proportional to the RBC count
- MCV: Normocytic (normal size RBC) Macrocytic (larger size RBC) when regeneration is rapid.
  Microcytic (smaller size RBC)—in chronic cases
- MCHC: Normochromic (normal Hb), hypochromic (decreased Hb) in chronic cases
- RDW: Increased
- Reticulocytes: Increased usually 5–20% (maybe as high as 70–90%. Normal range: 0.2–2%)
- Low Hb values and high reticulocyte count are important evidence of hemolytic anemia.
- Nucleated red blood cells: Normally they are absent but commonly found in hemolytic anemia.
- Normoblasts increase in hemolytic anemia.
  - Morphological changes in erythrocytes
- Presence of spherocytes (may be due to congenital or acquired spherocytosis)
- Acanthocytes are present in spur-cell anemia.
- Target cells are seen in thalassemia
- Elliptocytes are seen in hereditary elliptocytosis
- Sickle cells are seen in sickle cell anemia and in anemias where HbS is associated with other abnormal hemoglobins.
  - Confirmed by electrophoresis of hemoglobin

- Increase in HbA_2 is observed in β-thalassemia and β-unstable hemoglobinopathy.
- Increase in HbF is observed in β-thalassemia major trait and also in sickle cell disease.
  - Confirmed by sickling test
- It is positive in the HbS trait, homozygous Hb-S disease, Hb-S/C disease, Hb-S/β thalassemia
  - Biochemical tests: These tests give evidence of increased hemolysis.
- Serum bilirubin, total and indirect, both types are increased.
- SGOT and SLDH: Increased

Competency achievement: The student should be able to:

PA16.3: Describe the pathogenesis and clinical features and peripheral blood picture of sickle cell anemia and thalassemia

LAQ: Describe the pathogenesis and clinical features and peripheral blood picture of Sickle cell anemia and thalassemia.

Ans: Sickle cell anemia: Sickle cell anemia (SCA) is an inherited autosomal recessive disease characterized by the presence of homozygous hemoglobin S (HbS). It is caused by a single nucleotide mutation that substitutes glutamic acid for valine in the sixth position of the β-globin gene.

Pathogenesis and clinical features: During hypoxic conditions, the red blood cell becomes sickled and the resulting change in structure restricts circulation obstructing the blood flow within the capillaries and early destruction of the cell.

Clinical manifestations of SCA: Clinical manifestations of SCA vary from asymptomatic, mild to severe forms that are associated with high mortality rates. Clinical manifestations usually appear after three months of age, when the concentration of fetal hemoglobin (Hb F) decreases.

The abnormality in the structure of HbS leads to the polymerization of Hb molecules in hypoxia, which causes a distortion of the red blood cell into a sickle shape. Due to polymerization and sickling, the viscosity of blood increases, and it affects blood circulation.

In the heterozygous (AS) condition, 50% Hb in red blood cells is normal; hence the sickle cell trait may not produce clinical symptoms.

In SCA increased breakdown of red blood cells may lead to hemolytic anemia and increased metabolism of protoporphyrin. The concentration of indirect bilirubin may increase in the blood leading to prehepatic jaundice.

A definitive treatment to cure SCA is not currently available. Existing therapies are based on symptom management. These therapies are comprised of pain management, prevention of infections, appropriate nutrition, hydration, and precautions against adverse weather conditions.

Thalassemias: Thalassemias comprise a heterogeneous group of hereditary disorders of hemoglobin synthesis. The thalassemias are characterized by a reduced rate of production of normal hemoglobin due to the absence or decreased synthesis of one or more types of globin polypeptide chains (α or β chains). These are a heterogeneous group of disorders with a genetically determined reduction in the rate of synthesis of one or more types of the normal hemoglobin polypeptide chain. This results in a decrease in the amount of hemoglobin. It is a common disorder with a widespread geographical distribution.

There are two main groups of thalassemia, one affecting the synthesis of β-chains (β-thalassemia) and the other affecting the synthesis of α-chains (α-thalassemia).

The β-Thalassemias

In the case of β-thalassemias, the genetic mutation leads to a decreased rate of β-chain synthesis with a reduction in the amount of Hb-A in the red cell. This results in microcytic hypochromic anemia. The following are two main types of β-thalassemia.

β-Thalassemia Minor (Trait)

It is characterized by a moderate reduction in β-chain synthesis as directed by a β-thalassemia gene (inherited from one carrier parent). Clinically, it is a mild disorder with a little or no anemia. Usually, there is a normal expectancy without any significant symptoms. The spleen may be palpable. This condition may be detected only in a routine hematological screening test (often in pregnancy).

The following are hematological findings in β-thalassemia minor:

- The hemoglobin level is usually normal or mildly reduced.
- The red cell count is often normal (or maybe slightly increased).
- MCV and MCH are reduced
- MCHC is marginally reduced or normal
- Examination of blood film indicates mild red cell anisocytosis, microcytosis, and hypochromia with variable numbers of target cells.
- Red cell distribution width (RDW) is usually normal.
- Osmotic fragility test indicates increased resistance to hemolysis.
- HbA2: Increased (as seen by electrophoretic or chromatographic techniques).
- Serum iron and serum ferritin: Normal or increased (may be due to increased absorption of iron in the alimentary tract).
- Bone marrow appears cellular with erythroid hyperplasia.

β-Thalassemia Major

This condition occurs on average in one in four offspring if both parents are carriers of the β-thalassemia trait. In this condition, either small amounts of the beta chain are produced (β^+), or there is no synthesis of the beta chain (β°). In the absence of a beta chain, excess alpha chains are synthesized. These precipitate in erythroblasts and red cells and cause hemolysis, leading to hemolytic anemia. Synthesis of γ-chains, and its combination with α-chains results in the increased level of HbF (which varies from 20–90%).

Pathophysiology

1. Development of anemia stimulates the production of erythropoietin.
2. Hepatosplenomegaly is observed due to hyperfunction of the spleen.
3. Iron overload is observed due to hemolysis of red cells, increased absorption of iron from the gastrointestinal tract, and repeated transfusions given to these patients.
4. Many individuals present with severe anemia early in life and remain transfusion dependent for their entire lives.
5. Some have variable degrees of anemia and may require transfusion intermittently.
6. Erythroid hyperplasia leads to medullary expansion with facial deformity and osteoporosis
7. Extramedullary hematopoiesis results in enlargement of the liver and spleen
8. Excessive breakdown of red blood cells leads to hemolytic anemia. The hemoglobin from the destroyed red cells is liberated into the plasma. Hemoglobin appears in the urine if the absorptive capacity of the tubules is exceeded.
9. Due to the increased breakdown of red blood cells and metabolism of protoporphyrin, the concentration of indirect bilirubin increases in the blood leading to prehepatic jaundice.

The following are blood picture and laboratory findings in Thalassemia major:

- The anemia is usually severe.
- The red blood cells show marked anisocytosis and poikilocytosis.
- Tear drop cells and target cells are seen.
- Hypochromia is a striking feature.
- MCV and MCH are significantly decreased.
- MCHC is decreased.
- Normoblasts are present.

- Polychromasia and punctate basophilia are usually present.
- Reticulocyte count is increased.
- The white blood cell count is occasionally increased.
- Osmotic fragility test indicated an increased resistance to hemolysis.
- Serum indirect bilirubin is slightly increased.
- Serum iron and ferritin are elevated.
- Serum uric acid is frequently elevated.

Competency achievement: The student should be able to:

PA25.1: Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, distinguish between direct and indirect hyperbilirubinemia

BAQ: Describe normal bilirubin metabolism.

Ans: The following are the main steps of normal bilirubin metabolism (Fig. 13.7):

- Erythrocytes at the end of their lifespan are destroyed in the reticuloendothelial system. This amounts to the liberation of about 1% hemoglobin from the destroyed red blood cells.
- Globulin is separated from hemoglobin, and the porphyrin ring is opened.
- The released iron part enters the iron stores or may be used for further hemoglobin synthesis.
- Green-colored biliverdin forms first from the non-iron-containing residue of hemoglobin (i.e. protoporphyrin).
- Biliverdin gets reduced to yellow-colored bilirubin. Although most of the bilirubin is thus derived from hemoglobin, 20% also forms from the breakdown of tissue cytochromes and other hemoproteins. This bilirubin is water-insoluble and called indirect bilirubin.
- Indirect bilirubin circulates in the plasma bound to albumin.
- In the liver, bilirubin is separated from albumin and is conjugated with glucuronic acid to form water-soluble direct bilirubin (bilirubin glucuronide or conjugated bilirubin). This reaction is catalyzed by the enzyme bilirubin UDP glucuronic transferase. Direct bilirubin is water soluble, and it is excreted in the urine.
- Conjugated direct bilirubin is excreted into the biliary canaliculi, and then through the bile duct, it passes to the intestine.
- In the large intestine, it is reduced by bacterial action to a group of colorless chromogens, including urobilinogen.

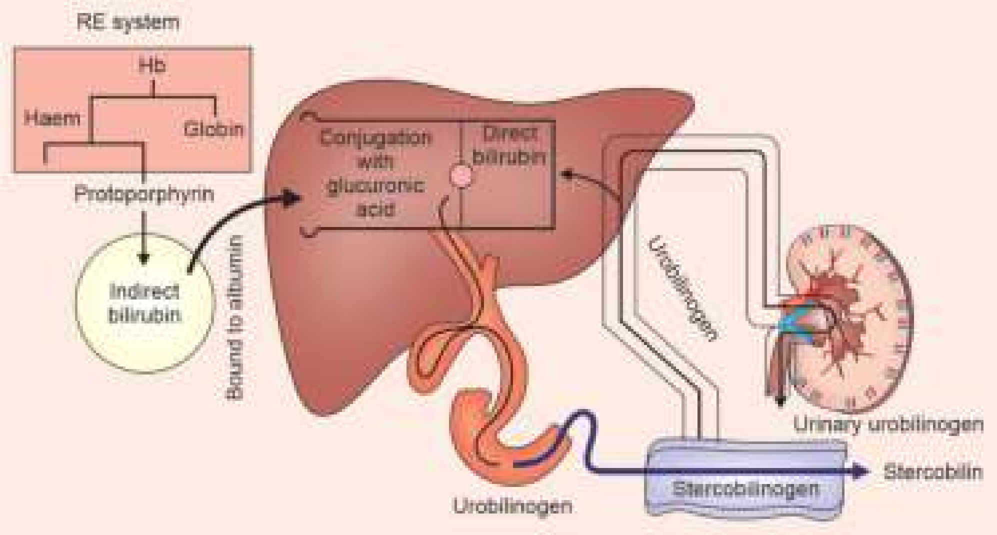


Fig. 13.7: Normal hemoglobin metabolism

- A small fraction of urobilinogen is absorbed into the portal circulation. Partly it is excreted in bile, while the remainder is excreted by the kidneys.
- Most of the urobilinogen is excreted in the feces, where it is oxidized by the air to the pinkish-brown urobilin.
- It has been estimated that 1 g of hemoglobin yields 35 mg of bilirubin.
- The liver of an adult has the reserve capacity to conjugate and excrete 5–10 times its normal load of bilirubin.
- In the case of a normal person, total bilirubin is generally < 1 mg/dl. In urine, chemically detectable bile pigments are not present, but a small quantity of urobilinogen is generally present in a freshly voided urine sample (Fig.13.7).

SAQ: What is the difference between total, direct, and indirect bilirubin?

Ans: Direct bilirubin is water-soluble bilirubin

Indirect bilirubin is water-insoluble bilirubin

Total bilirubin is the sum of direct bilirubin and indirect bilirubin (i.e. direct bilirubin + indirect bilirubin).

SAQ: Write reference ranges (normal ranges) of serum total bilirubin, direct bilirubin and indirect bilirubin.

Ans:	**Reference range** (Normal range)
Serum total bilirubin:	<1 mg%
Direct bilirubin:	<0.5 mg%
Indirect bilirubin:	<0.5 mg%

SAQ: Write a definition of jaundice (icterus).

Ans: Jaundice or icterus refers to the yellow pigmentation of the skin, conjunctiva, and mucous membranes by bilirubin. Clinically it becomes apparent when the serum total bilirubin concentration exceeds 2 mg/dl.

SAQ: Why there are incidences of increased serum bilirubin in a newborn?

Ans: The enzymes responsible for conjugation are not fully active at birth, so the liver of a newborn cannot excrete its normal bilirubin load. This load may be increased due to the excessive breakdown of erythrocytes. Jaundice before 24 hours of age is abnormal, but moderate hyperbilirubinemia (< 5 mg/dl) within the first week may not be pathological.

SAQ: Why chemically detectable bilirubin is not present in the feces of an adult, while it is present in the feces of a newborn?

Ans: Bilirubin is not present in the feces of a normal adult, since it is converted to urobilinogen by normal-flora bacteria in the intestine.

In the case of a newborn, the mechanism of bacterial reduction of bilirubin takes some months to develop fully. Hence in the feces of a newborn, bilirubin is present, and urobilinogen is absent in both feces and urine.

LAQ: What is hyperbilirubinemia? Describe the pathophysiology, recommended laboratory tests, and expected test values in indirect hyperbilirubinemia (pre-hepatic jaundice).

Ans: Hyperbilirubinemia means an increase in serum bilirubin >2 mg. Hyperindirect bilirubinemia means an increase in serum indirect bilirubin >1 mg. It will take place when excessive breakdown of red blood cells is observed in hemolytic anemia.

Jaundice may be due to the increased formation of indirect bilirubin; than the normal liver can convert it into direct bilirubin and excrete it. There is no hepatic damage.

The liver is not affected in the early development of hyper-indirect bilirubinemia. Hence, liver enzymes such as SGPT and alkaline phosphatase are normal. Moderately elevated SGOT (RBC origin) are observed, due to excessive destruction of red blood cells.

Laboratory tests: The following laboratory tests are performed on the serum and urine of a patient:

Serum tests: Total bilirubin, direct bilirubin, indirect bilirubin

Urine tests: Bile salts, bile pigments, urobilinogen

Blood: Total reticulocytes

Feces: Color

General laboratory observations in hemolytic jaundice:

1. Serum: Icteric (yellow colored).
2. Serum tests:
   a. Total bilirubin : Increased
   b. Direct bilirubin : Normal
   c. Indirect bilirubin: Increased
   d. SGPT: Normal
   e. SGOT: Moderately increased
   f. Alkaline phosphatase: Normal
3. Urine:
   a. Freshly voided: Normal color
   b. On standing: Becomes dark due to the formation of high urobilin from increased urobilinogen
4. Urine tests:
   a. Bile salts: Absent
   b. Bile pigments: Absent
   c. Urobilinogen: Very high
5. Stools dark colored due to the formation of urobilin from increased urobilinogen
6. Hemoglobin: Reduction in hemoglobin concentration of blood
7. Reticulocytes: Increased

LAQ: Describe the pathophysiology, recommended laboratory tests, and expected test values in direct hyperbilirubinemia (hepatic jaundice and post-hepatic).

Ans: Hyperdirectbilirubinemia means an increase in serum direct bilirubin >1 mg. Jaundice may be due to the increased formation of direct as well as indirect bilirubin.

The main reasons for hyper direct bilirubinemia are:

1. Due to infective hepatitis (by viral, bacterial diseases). The related clinical condition is hepatic.
2. Due to cholestasis, i.e. failure of normal excretion of bile in the intestine, mainly due to obstruction by gall stones or by cancerous growth.

The normal liver cannot convert a high load of direct bilirubin into indirect bilirubin.

Hence, direct bilirubin increases, and indirect bilirubin also increases due to the accumulated load of total bilirubin in the liver, leading to jaundice.

Laboratory tests: For the diagnosis of hepatic condition:

The following laboratory tests are performed on the serum and urine of a patient in hepatic as well as post-hepatic conditions:

Serum tests: Total bilirubin, direct bilirubin, indirect bilirubin

Urine tests: Bile salts, bile pigments, urobilinogen

General laboratory observations in hepatic jaundice:

1. Serum: Icteric (yellow colored).
2. Serum tests:
   a. Total bilirubin: Increased
   b. Direct bilirubin: Increased
   c. Indirect bilirubin: Increased
   SGPT: High or very high
   SGOT: High or very high
   Alkaline phosphatase: Moderately increased
3. Urine:
   a. Freshly voided: Dark yellow colored
   b. On standing: Becomes dark due to the formation of high urobilin from increased urobilinogen
4. Urine tests:
   a. Bile salts: Present
   b. Bile pigments: Present
   c. Urobilinogen: Increased

General laboratory observations in post-hepatic jaundice:

1. Serum: Icteric (yellow colored).
2. Serum tests:
   a. Total bilirubin : Increased
   b. Direct bilirubin : Increased
   c. Indirect bilirubin: Increased
   SGPT: High
   SGOT: High
   Alkaline phosphatase: Very high
3. Urine:
   a. Freshly voided: Dark yellow colored
   b. On standing: Becomes dark due to the formation of high urobilin from increased urobilinogen
4. Urine tests:
   a. Bile salts: Present
   b. Bile pigments: Present

Multiple Choice Questions

Q1. Which of the following dietary components needed for normal development of red blood cells?
A. Calcium and phosphorus
B. Iron and magnesium
C. Vitamin B_{12} and folic acid
D. Proteins

Q2. Normal lifespan of red blood cells is about
A. 120 days
B. 240 days
C. 10 days
D. 360 days

Q3. Which of the following clinical conditions is directly related to excessive destruction of red blood cells?
A. Aplastic anemia
B. Anemia
C. Sickle cell anemia
D. Megaloblastic anemia

Q4. Which of the following pathways is responsible for 90% of red blood cells glucose requirement?
A. HMS
B. Citric acid cycle
C. Gluconeogenesis
D. Embden-Meyerhof

Q5. Major regulator of human erythropoiesis is
A. Vitamin B_{12}
B. Folic acid
C. Erythropoietin
D. Vitamin D

Q6. All these may lead to bone marrow depression *except*
A. X-ray exposure
B. Vitamin A deficiency
C. Antifolic acid drugs
D. All of the above

Q7. All these are required for the synthesis of hemoglobin *except*
A. Amino acids
B. Iron
C. Copper
D. Sodium

Q8. Excretion of which of the following is increased in porphyrias?
A. Bilirubin
B. Aminolevulinic acid
C. Porphobilinogen
D. B and C

Q9. Porphyrias are caused due to deficiency of some of the following enzymes *except*
A. ALA synthase
B. Glucuronyltransferase
C. Uroporphyrinogen I synthase
D. Coproporphyrinogen oxidase

Q10. Which of the following enzymes is responsible to convert water-insoluble bilirubin into water-soluble bilirubin in the liver?
A. Glucuronyltransferase
B. ALA synthase
C. Uroporphyrinogen I synthase
D. ALA dehydrogenase

Q11. HBA is formed of two α chains and two
A. γ chains
B. δ chains
C. β chains
D. ε chains

Q12. HBF is formed of two α chains and two
A. γ chains
B. δ chains
C. β chains
D. ε chains

Q13. Percent of normal HBA is about
A. 96%
B. 50%
C. 80%
D. 45%

Q14. When fully saturated, 1 g of hemoglobin holds approximately
A. 5.0 ml oxygen
B. 0.5 ml oxygen
C. 2.5 ml oxygen
D. 1.34 ml oxygen

Q15. Normally blood methemoglobin levels are
A. 2–5%
B. < 1%
C. 5–10%
D. > 10%

Q16. Taut form of Hb is favored by all of these *except*
A. High pH
B. Low pH
C. High CO_2
D. High 2,3 BPG

Q17. Which of the following favors the binding of oxygen to Hb in the lungs?

A. High CO_2
B. Low 2,3-BPG
C. High CO_2
D. A and B

Q18. Which of the following gases has about 200 times stronger affinity to Hb than that of oxygen?

A. CO_2
B. CO
C. N_2
D. H_2S

Q19. Bohr effect means

A. Decrease in hemoglobin's affinity for oxygen by binding of acid (protons) and CO_2
B. Increase in hemoglobin's affinity for oxygen by binding of acid (protons) and CO_2
C. No change in hemoglobin's affinity for oxygen by binding of acid (protons) and CO_2
D. B or C

Q20. Fetal hemoglobin binds with oxygen

A. With the same affinity as HBA
B. With higher affinity than HBA
C. With less affinity than HBA
D. A or C

Q21. Major constituent protein of the cytoskeleton of red blood cells is

A. Ankyrin
B. Glycophorin
C. Spectrin
D. Collagen

Q22. All there are housekeeping enzymes of red blood cells *except*

A. Catalase
B. Glutathione peroxidase
C. Superoxide dismutase
D. Pyrimidine 5-nucleotidase

Q23. Abnormality in HBS is due to:

A. Substitution of normal glutamic acid in primary structure by lysine
B. Substitution of normal glutamic acid in primary structure by valine
C. Substitution of normal glutamic acid in primary structure by glutamine
D. Substitution of normal glutamic acid in primary structure by pyruvate

Q24. A 26-year-old woman had complaints of weakness and lethargy. Her blood hemoglobin was 8.3 g/dl (Normal: 13–16 g/dl). In microscopic stained blood smear examination a large number of immature red blood cells were observed. In urine high amount of FIGLU (a metabolite of histidine) was detected. The type of hematological disorder she was suffering

A. Iron deficiency anemia
B. Thalassemia major
C. Megaloblastic anemia
D. Leukemia.

Q25. An 8-year-old boy presented with loss of weight, headache, weakness, lethargy, and yellow discoloration of the skin and yellow eyes. His blood hemoglobin was 6.8 g/dl (Normal: 12–14.5 g/dl). In microscopic stained blood smear examination a large number of abnormal red blood cells were observed (hypochromia, microcytosis, poikilocytosis, etc).

A. Iron deficiency anemia
B. Thalassemia major
C. Megaloblastic anemia
D. Leukemia

Q26. The type of hematological disorder he was suffering from

A. Iron deficiency anemia
B. Thalassemia major
C. Megaloblastic anemia
D. Leukemia

Answers

1. C **2.** A **3.** A **4.** D **5.** C **6.** B **7.** D **8.** D 9. B 10. A
11. C **12.** A **13.** A **14.** D **15.** B **16.** A **17.** D **18.** B **19.** A **20.** B
21. C **22.** D **23.** B

24. C: Megaloblastic anemia. In histidine metabolism, histidine is converted to formiminoglutamic acid (FIGLU), which is converted to glutamic acid by the enzyme glutamate for aminotransferase, which requires folic acid as a cofactor. Folic acid deficiency leads to an inability to degrade a FIGLU to glutamic acid. Hence excessive FIGLU excretes in the urine.

25. B: Thalassemia major. Very low hemoglobin, and jaundice (as indicated by yellow skin and eyes, with a large number of microcytes, podocytes, and hypochromia, indicate: Thalassemia major.

26. (B)

Case Studies

Case 1: A 35-year-old woman presented with loss of weight, weakness, fatigue, palpitations, and dyspnea (shortness of breath) on exertion. Her blood test report values were as follows:

	Reference range (Normal range)
Hemoglobin: 5.8 g/dl	12–16 g/dl
PCV: 21%	36–48%
MCV: 71.7 fL	82–92 fL
MCH: 19.1 pg	27–32 pg
MCHC: 26.7 %	32–36 %

Stained peripheral blood smear microscopic observations:

Presence of a significant number of hypochromic and microcytic RBCs:

Hypochromia: +++, Microcytosis ++, Anisocytosis (cells with variable sizes) +++

1. What is the diagnosis?

Ans: Iron deficiency anemia (IDA). Hypochromic microcytic anemia. Significantly decreased Hb, PCV, MCV, MCH, and MCHC indicate IDA.

2. What is the biochemical basis of the disease?

Ans: Deficiency of iron leads to poor development of red blood cells.

3. What is the first-line treatment?

Ans: It is necessary to find out the primary cause of iron deficiency and treatment is given accordingly. Once the primary cause is diagnosed and treated, care is taken to provide sufficient iron with a diet rich in iron (meat, egg yolk, fish, nuts, legumes, dates, leafy green vegetables, dry fruits, etc.).

Oral therapy includes iron in tablets or syrup in the form of hemoglobin preparations, and 10 ml BD after meals up to six months. It is necessary to determine complete blood count (CBC) after every two months to follow the effects of the therapy and recovery status.

4. What additional blood tests are suggested?

Ans: Serum total iron, serum total iron binding capacity (TIBC), and serum ferritin.

BAQ: Show horizontal integration of symptoms and test reports of **Case 1** with anatomy, physiology, and nutrition

Ans: Horizontal integration with anatomy

Affected development of red blood cells

Loss of body weight, effect on general growth

Horizontal integration with physiology

Decrease in the oxygen-carrying capacity of RBCs.

Horizontal integration with nutrition

Feeding of adequate iron-rich diet and food supplements to cure iron-deficiency anemia.

BAQ: Show vertical integration of symptoms and test reports of **Case 1** with pharmacology, microbiology, and preventive medicine

Ans: Vertical integration with pharmacology

Study and use of appropriate drugs to treat IDA.

Vertical integration with microbiology

Study of gastrointestinal microbial and parasitic infections that may cause intestinal bleeding.

Vertical integration with preventive medicine

Study of education on good food habits to prevent IDA.

Case 2

A 63- year-old man presented with lethargy, fatigue, pallor, and weakness. His blood test report values were as follows:

	Reference range (Normal range)
Hemoglobin: 8.5 g/dl	13–17 g/dl
PCV: 31.6%	36–48%
MCV: 101 fL	82–92 fL
MCH: 34.6 pg	27–32 pg
MCHC: 37.3%	32–36%

Stained peripheral blood smear microscopic observations:

Presence of a significant number of hypochromic and macrocytic RBCs: Hypochromia: ++, macrocytosis ++

1. What is the diagnosis?

Ans: Megaloblastic anemia. Hypochromic macrocytic anemia

Very low hemoglobin, hypochromia, macrocytosis, and an increase in MCV, MCH, and MCHC, indicate that the patient is suffering from megaloblastic anemia.

2. What is the biochemical basis of the disease?

Ans: Deficiency of folic acid or vitamin B_{12}, which are required for the normal development of red blood cells.

Q.3. What is the first-line treatment?

Ans: It depends upon the basic cause of megaloblastic anemia. It may be due to folic acid deficiency or due to pernicious anemia. In any case, vitamin B complex tablet 1 OD, containing 5 mg of folic acid and 1000 µg of vitamin B_{12} is prescribed with other vitamins and food supplements, till blood levels of folic acid and vitamin B_{12} are restored, with improvements in the CBC report. B-complex vitamin tablets, containing 5 mg folic acid and 15 µg vitamin B_{12}, continued later on, one per day, with good sources of folic acid. The rich sources of folic acid are leafy green vegetables (spinach, asparagus, turnip greens), cereals, whole grains, eggs, liver, kidney, and yeast.

For the management of pernicious anemia, one vitamin B_{12} injection (500 µg), per day for a week is recommended with good food supplements and appropriate follow-up, till serum levels of vitamin B_{12} are restored. The rich sources of vitamin B_{12} are milk, fish, liver, kidney, pork, egg, chicken, curd, etc. During the follow-up phase, one tablet of vitamin B_{12} (1000 µg) per week is suggested lifelong.

4. What additional tests are suggested?

Ans: Serum folic acid, red blood cell folic acid and serum vitamin B_{12}.

BAQ: Show horizontal integration of symptoms and test reports of **Case 2** with anatomy, physiology, and nutrition.

Ans: Horizontal integration with anatomy

Affected development of red blood cells

Horizontal integration with physiology

Decrease in the oxygen-carrying capacity of RBCs.

Horizontal integration with nutrition

Feeding adequate folic acid, vitamin B_{12}-rich diet, and food supplements to manage megaloblastic anemia.

BAQ: Show vertical integration of symptoms and test reports of **Case 2** with pharmacology, microbiology, and preventive medicine

Ans: Vertical integration with pharmacology

Study and use of appropriate drugs to treat megaloblastic anemia.

Vertical integration with microbiology

Study of gastrointestinal microbial infections (such as *Helicobacter pylori*) that may cause deficiency of vitamin B_{12}.

Vertical integration with preventive medicine

Preventive medicine: Study of education of good food habits and hygiene to prevent vitamin deficiency and microbial infections, respectively.

Case 3: A 4-year-old girl was admitted to the hospital because she was suffering from loss of weight, recurrent fever, anorexia, and diarrhea. Her blood test reports were as follows:

	Reference range (Normal range)
Hemoglobin: 5.9 g/dl	11.5–14.5 g/dl
PCV: 20.7%	36–48%
MCV: 72.2 fL	82–92 fL
MCH: 20.6 pg	27–32 pg
MCHC: 28.5 %	32–36 %

Stained peripheral blood smear microscopic observations:

A large number of hypochromic red blood cells (Hypochromia: ++++).

A large number of microcytes (microcytosis: ++)

A large number of poikilocytes (red blood cells with abnormal shapes)

(Poikilocytosis: ++)

Target cells ++

1. What is the diagnosis?

Ans: Thalassemia major. Very low hemoglobin, hypochromia, microcytosis, poikilocytosis, presence of a significant number of target cells and decrease in PCV, MCV, MCH, and MCHC, indicate that the patient is suffering from thalassemia major. In thalassemia minor, all these blood parameters do not show decrease comparable with the blood test reports of Case 3.

2. What is the biochemical basis of the disease?

Ans: Thalassemia syndromes are characterized by the genetic defects in the synthesis of one or more of the globin chains that form the hemoglobin tetramer. In β thalassemia the inadequate production of β chains of hemoglobin leads to a reduction in HbA in red blood cells. This leads to an increase in γ and δ chains, with an increase in HbF and HbA_2. These γ and α chains aggregate and interfere with erythroid cell maturation leading to ineffective erythropoiesis.

3. What is the treatment?

Ans: Treatment is supportive. Thalassemia major cases often require frequent blood transfusions. Frequent blood transfusions cause a buildup of iron in the blood, which can damage various organs. Hence chelation therapy is also given to remove excess iron from the blood.

Bone marrow transplant (stem cell transplant) can be one option in some cases of thalassemia major.

4. What additional laboratory tests are suggested?

Ans: Hb-electrophoresis to find out the increase in HbA_2 and HbF. DNA PCR test to confirm the diagnosis.

BAQ: Show horizontal integration of symptoms and test reports of **Case 3** with anatomy and physiology.

Ans: Horizontal integration with anatomy

Affected bone marrow and development of red blood cells

Horizontal integration with physiology

Decrease in the oxygen-carrying capacity of RBCs.

BAQ: Show vertical integration of symptoms and test reports of **Case 3** with pharmacology, general medicine, and preventive medicine.

Ans: Vertical integration with pharmacology

Study and use of appropriate therapy to manage thalassemia major.

Vertical integration with general medicine

Study of management of thalassemia major.

Vertical integration with preventive medicine

Preventive medicine: Study and promotion of education related to the prevention of cases of thalassemia major by encouraging laboratory tests on every individual before marriage. There is a 50% chance of a child being born with a thalassemia major, if both parents have thalassemia minor.

Chapter

14

Nutrition

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

Nutrition is the science or study that deals with food and nourishment. It is a process of eating food and utilizing food substances. In general, humans can survive for two to eight weeks without food, depending on stored body fat. Without water humans usually can survive `1for three or four days. Human nutrition is the provision to humans to obtain the materials necessary to support life. Food generates energy and supplies materials used in body tissues and processes.

There are seven major classes of nutrients: Carbohydrates, fats, dietary fiber, minerals, protein, vitamins, and water. Calories required for growth and maintenance of good health are obtained by eating carbohydrates, fats, and proteins. Minerals are useful in several ways such as iron for hemoglobin; calcium for bones, teeth, and cellular processes; sodium and potassium to regulate homeostasis, iodine to produce thyroid hormones, etc. Fiber is not broken down chemically in the body but aids digestion, lowers blood cholesterol, and may help to prevent some cancers and hypertension. Different amounts of these nutrients exist in different foods and a balanced diet ensures an adequate supply of essential nutrients. Inadequate nutrient intake or absorption leads to malnutrition and disease.

The human body contains chemical compounds, such as water, carbohydrates (sugars, starch, and fiber), amino acids (in proteins), fatty acids (in lipids), and nucleic acids (in DNA and RNA). These compounds in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, iron, zinc, magnesium, manganese, etc. All of these chemical compounds and elements occur in various forms in the human body. Most balanced foods contain a mix of most of the required chemical compounds and nutrient classes. Some nutrients can be stored internally (e.g. carbohydrates in the form of glycogen and lipids in adipose tissue), while others are required more or less continuously. Poor health can be caused by a lack of required nutrients, or in extreme cases, too much of a required nutrient. For example, both salt and water will cause illness or even death in too large amounts.

About 70% of the non-fat mass of the human body is made of water. To function properly, the body requires an adequate intake of water per day to avoid dehydration. The precise amount of water intake depends on the level of activity, temperature, humidity, and other factors. With physical exertion and heat exposure, water loss increases, and daily fluid needs will eventually increase as well.

The nutrient classes can be categorized as either macronutrients (required in relatively large amounts) or micronutrients (required in smaller quantities). The macronutrients are proteins, carbohydrates, fats, water, fiber, and water. The micronutrients are minerals and vitamins. Other micronutrients include antioxidants and phytochemicals which also influence some body systems.

The macronutrients (excluding fiber and water) provide structural material. From amino acids proteins are built, and from lipids cell membranes and some signaling molecules are built. Energy for the anabolic processes is mainly derived from carbohydrates. Some of the structural materials can be used to generate energy internally. Energy is measured in Joules or kilocalories. Carbohydrates and proteins provide approximately 4 kcal of energy per gram, while fats provide 9 kcal per gram. Although vitamins, minerals, and water do not provide energy, these are required for several energy-producing chemical reactions.

The human body consists of elements and compounds obtained after eating food which are ingested, digested, absorbed, and circulated through the bloodstream to feed the cells of the body.

The primary technology used to maintain freshness is to store food at cold temperatures. Similarly, many more technologies have been invented to allow foods to last longer without becoming spoiled. These latter technologies include pasteurization, autoclavation, drying, salting, and separation of various food components. All these techniques appear to alter the original nutritional contents of food. Pasteurization and autoclavation improve the safety of many common foods. But these technologies are responsible to destroy vitamins and minerals. Because of reduced nutritional value, processed foods are often 'enriched' or 'fortified' with some of the most critical nutrients (usually certain vitamins) that were lost during processing. Nonetheless, processed foods tend to have an inferior nutritional profile compared to fresh food.

DIETARY COMPONENTS

Competency achievement: The student should be able to:

BI8.1: Discuss the importance of various dietary components and explain the importance of dietary fiber

BAQ: Discuss the importance of various dietary components and explain the importance of dietary fiber.

Ans: There are seven major classes of nutrients: Carbohydrates, fats, dietary fibers, minerals, proteins, vitamins, and water. Carbohydrates are the most abundant dietary constituents and also the chief source of energy. Fats are concentrated dietary source of energy, which contribute to about 20–50% of the energy requirement of the body. Although calories required for growth and maintenance of good health are obtained by eating carbohydrates, proteins, and fats, proteins are regarded as "body-building components" of food.

Minerals are useful in several ways as iron is required for hemoglobin synthesis; calcium for the normal formation of bones, teeth, and cellular processes; sodium and potassium to regulate homeostasis, iodine to produce thyroid hormones, etc.

Dietary fiber consists mainly of cellulose, a large carbohydrate polymer that is indigestible because humans do not have the required enzymes to disassemble it. There are two subcategories of fiber, soluble and insoluble fiber. Whole grains, fruits, and vegetables are good sources of dietary fiber. Fiber is important to digestive health. It helps in alleviating both constipation and diarrhea. Fiber provides bulk to the intestinal contents, and insoluble fiber especially stimulates peristalsis, i.e. the rhythmic muscular contractions of the intestines which move digested food along the digestive tract. Some soluble fibers form solutions of high viscosity; which is essentially a gel, that slows the movement of food through the intestines. Fiber is not broken down chemically in the body but aids digestion, lowers blood cholesterol and may be useful to prevent some cancers and hypertension. Different amounts of these nutrients exist in different foods and a balanced diet ensures an adequate supply of essential nutrients.

The human body contains chemical compounds, such as water, carbohydrates (sugars, starch, and fiber), amino acids (in proteins), fatty acids (in lipids), and nucleic acids, in the forms of DNA and RNA. These compounds in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, iron, zinc, magnesium, manganese, etc. All of these chemical compounds and elements occur in various forms in the human body. Most balanced foods contain a mix of most of the required chemical compounds and nutrient classes. Some nutrients can be stored internally (e.g., carbohydrates in the form of glycogen and lipids in adipose tissue), while others are required more or less continuously. Poor health can be caused by a lack of required nutrients, or in extreme cases, too much of a required nutrient.

About 70% of the non-fat mass of the human body is made of water. To function properly, the body requires an adequate intake of water per day to avoid dehydration. The precise amount of water intake depends on the level of activity, temperature, humidity, and other factors. With physical exertion and heat exposure, water loss increases and daily fluid needs eventually increase.

SAQ: Write two health benefits of food fibers.

Ans: Food fibers:

1. These are useful to maintain regular bowel movements.
2. Prevent rapid absorption of glucose in the gastrointestinal tract and thus, prevent blood sugar spikes.

BAQ: What are the nutrient classes and their importance?

Ans: The nutrient classes can be categorized as either macronutrients (required in relatively large amounts) or micronutrients (required in smaller quantities).

The macronutrients are proteins, carbohydrates, fats, water, and fiber. The micronutrients are minerals and vitamins. Other micronutrients include antioxidants and phytochemicals which also influence many body systems.

The macronutrients (excluding fiber and water) provide structural material. From amino acids proteins are built, and from lipids cell membranes and some signaling molecules are built. Energy for the anabolic processes is mainly derived from carbohydrates. Some of the structural materials can be used to generate energy internally.

SAQ: What are trans fatty acids? What are their effects on health?

Ans: Trans fatty acids (TFAs) are unsaturated fatty acids. These are not synthesized by mammals. These TFAs are characteristically produced during the industrial hydrogenation of plant oils. Since they are also produced in bacterial metabolism, ruminant fats (e.g., in milk) also contain about 4% trans fatty acids. Several interventional studies conducted on trans fatty acids indicate their correlation with an increase in inflammatory reactions leading to atherosclerosis and coronary heart disease, compared to the same amount of *cis* fatty acids. These studies also indicate that trans fatty acids, just like saturated fats, raise the LDL cholesterol and lower the HDL cholesterol.

BAQ: Why diet should contain a balance of omega-3 and omega-6 fatty acids and what are their dietary sources?

Ans: A healthy diet should contain a balance of omega-3 and omega-6 fatty acids, which helps to stimulate skin and hair growth, good bone health, regulation of metabolism, decrease inflammatory processes in the body, and also decrease risks for heart disease by maintaining low levels of atherogenic LDL.

The following are sources of 3-omega fatty acids:

Fish (tuna, salmon, mackerel, etc.), nuts and seeds (such as walnuts, flaxseed, etc, and plant oils (such as canola oil, flaxseed oil, soybean oil, etc.)

The following are sources of 6-omega fatty acids:

Meat, fish, eggs, poultry, corn, nuts, seeds, soybeans, safflower, and sunflower oils.

SAQ: How energy is measured? How much energy is provided by carbohydrates, protein, and lipids? What is the role of vitamins and minerals in deriving energy?

Ans: Energy is measured in Joules or kilocalories. Carbohydrates and proteins provide 17 kJ approximately (4 kcal) of energy per gram, while fats provide 37 kJ (9 kcal) per gram. Although vitamins, minerals, and water do not provide energy, these are required for several energy-producing chemical reactions.

SAQ: Enumerate methods for the preservation of food.

Ans: Food preservation methods include pasteurization, autoclavation, drying, salting, and separation of various food components.

SAQ: Why processed foods are fortified with critical nutrients?

Ans: Pasteurisation and autoclavation improve the safety of many common foods. However, these technologies are responsible to destroy vitamins and minerals. Because of reduced nutritional value, processed foods are often "enriched" or "fortified" with some of the most critical nutrients (usually with specific vitamins) that were lost during processing.

NUTRITIONAL FOOD VALUES

The nutritional value of food is the measure of a well-balanced ratio of the essential nutrients such as protein, carbohydrates, lipids, vitamins, and minerals; in items of diet with relation to the nutrient requirements of a person. Several nutritional rating systems have been used to rank food in terms of its nutritional value. International and national guidelines are available for consumers to provide information on optimal nutrient intake from their diets.

Respiratory Quotient (RQ)

BAQ: What is the respiratory quotient (RQ) of foodstuff and its significance?

Ans: RQ of foodstuff is the ratio of the volume of carbon dioxide produced to the volume of oxygen used in the oxidation of foodstuffs. The respiratory quotient (RQ) is a unitless number used in calculations of basal metabolic rate (BMR) when estimated from carbon dioxide production. Such measurements, like measurements of oxygen uptake, are forms of indirect calorimetry. RQ is measured using Ganong's Respirometer.

The RQ of carbohydrates is close to 1.0 since these are completely oxidized. RQ of fats is lower than 1.0 since they have a low oxygen content. For example, tristearin has RQ = 0.7. Since the chemical nature of proteins is variable, it is difficult to determine their RQ accurately. However, the average RQ of protein = 0.6. RQ of a mixed diet is dependent on the content of carbohydrates, protein, and fat and it is approximately = 0.8. In the case of organisms in metabolic balance, the RQ ranges from 1.0–0.7. RQ for an organism may rise above 1.0 if excess carbohydrates are converted to fats (which are deposited as food reserves).

RQ is used as an indicator of overeating (which may lead to obesity) or underfeeding (which may lead to loss of weight) and also dysfunction of various organs such as the lungs, liver, and kidneys that may lead to malnutrition.

Utilization of Energy

BAQ: Write a note on the utilization of energy.

Ans: Energy produced from food in the human body is used to maintain the body's essential functions such as growth, repair of cells, transportation of blood, respiration, muscular activities, activities of the nervous system, etc., and for performing physical activities such as work, exercise, and outdoor activities.

In the body, thermal energy is useful to maintain a constant body temperature, mechanical energy is useful to move, and electrical energy is useful in sending nerve impulses and fire signals to and from the brain. Energy is stored in foods and the body as chemical energy.

Energy can be utilized to meet the fuel demands by processes such as basal metabolic rate (BMR), and specific dynamic action (SDA), which increases metabolic rate following food ingestion and physical activity. In the case of an average individual with light work, 60% of the calories are used towards BMR, about 10% for SDA, and about 30% for physical activity. Additional energy is required during pregnancy, lactation, and growth.

Basal Metabolic Rate (BMR)

LAQ: Write a note on basal metabolic rate (BMR).

Ans: BMR is the energy expenditure by the body when at rest (but not asleep), under controlled conditions of thermal neutrality, measured about 12 hours after the last meal, and it depends on gender, weight, and height. BMR is the total number of calories the body requires for normal bodily functions (excluding activity factors). This includes keeping the heart beating, inhaling and exhaling air, digesting food, making new blood cells, maintaining body temperature, and every other metabolic process in the body.

BMR is the lowest when a person sleeps undisturbed. Other factors which influence BMR are physical activity, hormones, starvation, environmental conditions, disease states, and fever. Determination of BMR is important to calculate the calorie requirement of an individual and accordingly plan diets. For the determination of BMR, the individual should be in comfortable surroundings, awake, and at complete physical and mental rest. The BMR is determined by the apparatus of Roth or Benedict or by the Douglas bag method.

Basal metabolic rate is expressed as calories per square meter of body surface area per hour (cal/sq. m./hr).

Normal values:

- *Men:* 35–38 cal/sq. m./hr or 1600–1800 calories per day
- *Women:* 32–35 cal/sq. m./hr or 1400–1600 calories per day

The following are the main factors that influence BMR:

1. **Age:** With an increase in age, BMR decreases due to a natural decline in muscle mass and a decrease in physical activity.
2. **Gender:** Men have a higher BMR than females due to their higher muscle mass and low percentage of body fat.
3. **Body size:** Bodies with large sizes have a higher BMR than smaller bodies due to increased energy requirements in the maintenance of basic body functions.
4. **Hormones:** Hormones secreted by the thyroid gland affect BMR. Hormonal imbalances, during menopause, can affect BMR.
5. **Diet:** BMR increases following food intake and the increase remains for about two to three hours and it is dependent on size and type of food.

A prolonged increase in BMR may lead to loss of weight, as observed in fever, diseases such as cancer, hyperthyroidism, and also in pregnancy. A prolonged decrease in BMR may lead to gain of weight as seen in hypothyroidism and obesity.

In patients with cachexia, there is an increase in the rate of tissue protein catabolism and an increase in metabolic rate due to the release of cytokines in response to tumors and related clinical conditions.

Importance of determination of BMR: The determination of BMR is used to calculate the requirement of calories of a person or a patient and accordingly the planning of diet.

BAQ: Mention two methods for the determination of BMR and the normal range of BMR for men and women.

Ans:

1. The BMR is determined by the apparatus of Roth or by the Douglas bag method.
2. BMR also can be determined by Katch-McArdle formula (based on lean body weight):

   BMR (men and women) = 370 + (21.6 × lean mass in kg)

Example:

In the case of a female: Weight 120 lbs. (54.5 kilos), body fat percentage is 20% (24 lbs. fat, 96 lbs. lean), lean mass is 96 lbs. (43.6 kilos), BMR = 370 + (21.6 × 43.6) = 1312 calories

Normal values:

- Men: 35–38 cal/sq. m./hr or 1600–1800 calories per day
- Women: 32–35 cal/sq. m./hr or 1400–1600 calories per day

Specific Dynamic Action

Q: Write a note of specific dynamic action.

Ans: Specific dynamic action (SDA) refers to the increased metabolic rate experienced following ingestion of a meal. It is also known as the thermal action or calorigenic action of food. SDA of food is due to the energy required for digestion, absorption, transport, metabolism, and storage in the body. Food having high SDA is not a good source of energy. Hence, protein is not a good source of energy having 30 SDA and carbohydrates are a good source of energy having 5 SDA.

SDA for carbohydrate, fat, and protein is 5%, 13%, and 30% respectively. For example, for food containing 25 g protein the heat production is 100 cal (25 × 4). However, when 25 g protein is used in the body, 130 cal of heat is liberated. Hence SDA of protein is (130–100) = 30. Similarly, consumption of 100 cal fat results in 113 cal. Hence SDA of fat = 113–100 = 13. Consumption of 100 cal carbohydrate results in 105 cal, hence SDA of carbohydrates = 105–100 = 5.

In general, the specific dynamic action (SDA) effect of food is the increment in energy expenditure above the resting metabolic rate due to the cost of processing food for storage and use. It is one of the components of metabolism along with the resting metabolic rate, and the exercise component.

Physical Activity Level (PAL)

BAQ: Define the physical activity level (PAL) of a person. Give a tabular form of PAL for various lifestyles.

Ans: The physical activity level (PAL) is defined for a normal person as total daily energy expenditure (TDEE, in 24 hours), divided by basal metabolic rate (BMR).

PAL = TDEE, 24 hours/BMR

Physical activity of the body depends on the personal activity. It depends on the amount of energy needed for the intensity and duration of muscular activity. For light office work, BMR is observed to increase 30–40% and it increases 40–50% with moderate work, 50–60% with moderately heavy work, and 60–100% with very heavy work.

Table 14.1: Physical activity level (PAL) for certain lifestyles

Lifestyle	Example	PAL
Inactive	A sleeping person	<1.40
Sedentary	Person working in an office (mostly sitting)	1.40–1.69
Worker	A person who runs at least one hour/day	1.70–1.99
Very active	A person who swims for 2 hours/day Agricultural worker	2.0–2.40
Vigorously	Tennis player or cyclist active for 3–5 hours	>2.40

DETERMINATION OF NUTRITIVE VALUE ASSESSMENT OF PROTEINS

SAQ: Enumerate the methods for the determination of nutritive value assessment of proteins.

Ans: Nutritional value assessment of proteins is done by considering various aspects such as protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), and chemical score.

Protein Efficiency Ratio (PER)

SAQ: What is protein efficiency ratio (PER)? How PER is determined and what is its significance?

Ans: Protein efficiency ratio (PER) is based on the weight gain of a test subject divided by its intake of a particular food protein during the test period.

PER is determined by feeding 21-day-old albino rats with a 10% test protein diet and recording their weight gain after four weeks. PER is calculated as follows:

PER = Gain in body weight (g)/Protein ingested (g)

PER measures the nutritive value of protein sources. Higher PER value of a protein is more beneficial to the animal.

Biological Value (BV)

LAQ: Write a note on the biological value (BV) of protein. Describe the methods and experiment for the determination of BV of protein.

Ans: Biological value (BV) is a measure of the proportion of absorbed protein from a portion of food that becomes incorporated into the proteins of the organism's body. It indicates how readily the broken protein in digestion and absorption can be used in protein synthesis in the cells of the organism.

Proteins are the major source of nitrogen in food, unlike carbohydrates and fats. This method assumes protein is the only source of nitrogen and measures the proportion of this nitrogen absorbed by the body and part of it is then excreted. The remainder must have been incorporated into the proteins of the body. A ratio of nitrogen incorporated into the body over nitrogen absorbed gives a measure of the BV.

For accurate determination of BV, it is necessary that:

1. The test organism must only consume the protein or mixture of proteins of interest (the test diet).
2. The test diet must not contain non-protein sources of nitrogen.
3. The test diet must be of suitable content and quantity to avoid the use of protein primarily as an energy source.

These conditions mean the tests are typically carried out over one week with strict diet control.

The following formula is used to determine the biological value (BV) of protein:

BV = (Nr/Na) × 100

Na = nitrogen absorbed in proteins on the test diet

Nr = nitrogen incorporated into the body on the test diet

However, direct determination of Nr is difficult. It is measured indirectly from nitrogen excretion in urine and fecal excretion of nitrogen. An estimate is used of the amount of urinary and fecal nitrogen excretion not coming from ingested nitrogen. This is done by substituting a protein-free diet and observing nitrogen excretion in urine or feces. Regarding these procedures, BV can be calculated as follows:

$$BV = \{[Ni - Ne(f) - Ne(u)]/[Ni - Ne(f)]\} \times 100$$

Ni = Nitrogen intake in proteins on the test diet.

$Ne(f)$ = (nitrogen excreted in feces while on the test diet) – (nitrogen excreted in feces not from ingested nitrogen).

$Ne(u)$ = (nitrogen excreted in the urine while on the test diet) – (nitrogen excreted in the urine, not from ingested nitrogen)

It is important to note the following points:

1. $Nr = Ni - Ne(f) - Ne(u)$
2. $Na = Ni - Ne(f)$
3. A BV of 100% indicates complete utilization of a dietary protein, i.e. 100% of the protein ingested and absorbed is incorporated into proteins into the body.
4. This method does not take into account the nitrogen that might be lost during the digestion process.

Determination of BV using albino rat: 21-day-old albino rats can also be used for the determination of BV. These are fed on a protein-free diet for 10 days. Urine and fecal nitrogen are determined after 10 days. These rats are then kept on a 10% protein diet for one month. At the end of the month, food nitrogen, and urine as well as fecal excretion of nitrogen is determined. BV is calculated by using the above-mentioned equation.

Net Protein Utilization (NPU)

SAQ: What is "the net protein utilization (NPU)"? Write the significance of NPU.

Ans: The net protein utilization (NPU) is the ratio of amino acids converted to proteins to the ratio of amino acids supplied. Experimentally, this value can be determined by determining dietary protein intake and then measuring nitrogen excretion. As a value, NPU can range from 1 to 0, with a value of 1 indicating 100% utilization of dietary nitrogen as protein. A value of 0 indicates that none of the nitrogen supplied was converted to protein. Certain foodstuffs, such as eggs or milk, are rated as 1 on an NPU chart.

$$NPU = \frac{\text{Nitrogen retained}}{\text{Nitrogen ingested}}$$

Chemical Score

BAQ: What is the chemical score of a protein? How chemical score of a protein is determined?

Ans: Chemical score of egg protein amino acids is considered as 100 and it is compared with the test protein.

The chemical score of a protein is determined by a comparison between the amount of essential amino acids in a dietary protein and the amount in a reference protein (e.g. egg protein). It is defined as the ratio between the quantity of the most limiting essential amino acid in the test protein to the quantity of the most limiting amino acid (essential amino acid) in the egg protein, expressed as a percentage. It is calculated by using the following formula:

Chemical score = mg of the limiting amino acid/g test protein/mg of the same amino acid/g egg protein × 100

Mutual Supplementation of Proteins

BAQ: Write a note on the mutual supplementation of proteins.

Ans: Foods containing protein are categorized as complete proteins or incomplete proteins. Complete proteins contain amino acids as required by humans. Meat, eggs, fish, cheese, poultry, milk, and yogurt are sources of complete proteins. Incomplete proteins are low-quality proteins. Legumes, cereals, grains, and vegetables are sources of incomplete proteins. A diet with only low-quality proteins is unable to supply amino acids and inhibits the ability of the body to synthesize proteins. It is important to ensure that all indispensable amino acids are received by the body. This is achieved by mutual supplementation.

In the case of a vegetarian, to receive a balanced distribution of essential amino acids, mutual supplementation is a nutritional strategy in which vegetable foods with low contents of amino acids (e.g. cereals) are eaten together with a food that is high in that same amino acid (e.g. pulses or legumes). Cereal proteins lack lysine and threonine, hence cereals should be taken with pulses (dal), which contain lysine and threonine. When two or more incomplete proteins are eaten, they compensate for the deficiency of essential amino acids. Following are a few recommended combinations:

- Potatoes and cheese
- Baked beans and bread
- Cornflakes and milk
- Pasta and cheese
- Rice with pulses (dal) and legumes
- Rice and wheat with tofu
- Bread and peanut butter
- Indian food like idli, dosa, and sambhar
- Lentils and bread

BAQ: Present in a tabular form the nutritive values in terms of PER, BV, NPU, and chemical score of egg, rice, meat and wheat.

Table 14.2: Nutritive value of food proteins (1)

Source of protein	PER	BV	NPU	Chemical score
Egg	4.5	94	90	100
Rice	2.2	68	60	60
Meat	2.7	75	76	70
Wheat	1.5	58	47	42

BAQ: Present in a tabular form nutritive values in terms of PER, BV, NPU, and chemical score of fish, groundnut, soybean, and Bengal gram.

Table 14.3: Nutritive value of food proteins (2)

Source of protein	PER	BV	NPU	Chemical score
Fish	3.0	85	70	60
Groundnut	1.7	55	45	44
Soybean	2.1	65	55	55
Bengal gram	1.7	58	47	45

LAQ: Write a note on a standard nutritional plan.

Ans: The first step in designing a personal nutrition plan is to calculate how many calories one burn in a day, i.e. total daily energy expenditure (TDEE). TDEE is the total number of calories that the body expends in 24 hours, including all activities. TDEE is also known as the "maintenance level" of an individual.

One method for TDEE calculation is to determine BMR using factors such as height, weight, age, and sex, then multiply the BMR by the physical activity factor related to sleep, table work, indoor work, out door work, exercise, etc. (Refer to Table 14.1), during 24 hours. BMR usually accounts for about two-thirds of total daily energy expenditure. It may vary dramatically from person to person depending on genetic factors. Those individuals who can eat any amount of food and never gain weight have inherited a naturally high BMR. It is very important to note that the higher the lean body mass (fat-free mass), the higher will be the BMR.

The average TDEE for women is 2000–2100 calories per day and the average TDEE for men is 2700–2900 calories per day. These are only averages; caloric expenditure can vary widely and is much higher for athletes or extremely active individuals. Some triathletes and ultra-endurance athletes may require as many as 6000 calories per day or more just to maintain their weight. This energy requirement is met by 40–60% carbohydrates, 30–40% lipids, and 10–15% protein.

Calorie requirements may also vary among otherwise identical individuals due to differences in inherited metabolic rates.

It is obvious then that one way to maintain lean body mass is to plan daily exercise regimens to increase BMR. In this manner, exercise programs help to lose body fat.

The United Healthcare/Pacificare nutrition guideline recommends the following foods per day to enhance general health (based on a 2,000 calorie diet): Grains: 170 g/day;

Vegetables: 2.5 cups/day; Fruits: 2 cups/day; Milk: 3 cups/day and Meat and beans; or Cereals and beans 140 g/day.

It is necessary to maintain a normal body mass index. Body mass index (BMI) is measured by the formula: Weight in kg divided by the square of height in meters. The result of this calculation is then interpreted as:

- <Below 18.5 = Underweight
- >18.5–24.9 = Normal or ideal weight
- >25–29.9 = Overweight.

Obesity is caused by consuming more calories than are expended, with many attributing excessive weight gain to a combination of overeating of "unhealthy" foods such as high fat, high sugar, high carbohydrate foods, and insufficient exercise.

Adjustment of caloric intake according to personal goal: Once the TDEE (maintenance level) is known, the next step is to adjust calories according to the personal primary goal. To keep weight at its current level, it is necessary to remain at a daily caloric maintenance level.

To lose weight, it is necessary to create a calorie deficit by reducing calories slightly below the maintenance level (or keeping calories the same and increasing activity above the current level). To gain weight it is necessary to increase the calories above the maintenance level. The only difference between weight gain programs and weight loss programs is the total number of calories required. A negative calorie balance is essential to lose body fat.

The calorie deficit can be created through diet, exercise, or preferably, a combination of both. Reduction in calories slows down the metabolic rate, decreases thyroid output, and causes loss of lean mass. A more individualized way to determine the safe calorie deficit would be to account for individual body weight or TDEE.

Example 1: If weight is 55 kg, TDEE is 2033 calories, and the calorie deficit to lose weight is 500 calories. The optimal caloric intake for weight loss is 2033 – 500 = 1533 calories.

Example 2: If the calorie deficit to lose weight is 20% of TDEE (20% × 2033 = 406 calories), the optimal caloric intake for weight loss = 1627 calories.

Positive calorie balance is essential to gain lean body weight. A general guideline for a starting point for gaining weight is to add approximately 300–500 calories per day onto individual TDEE. An alternate method is to add 15–20% in TDEE.

Example: If weight is 55 kg and TDEE is 2033 calories, the additional calorie requirement for weight gain is 15–20% = 305 – 406 calories. The optimal caloric intake for weight gain is 2033 + 305 – 406 = 2338 – 2439 calories.

It is not advisable to make any drastic changes to the diet all at once. After calculating total daily energy expenditure and adjusting it according to a personal goal, if the amount is substantially higher or lower than the current intake, then it is necessary to adjust the required calories gradually.

For example, if the optimal caloric intake is 1900 calories per day, and the actual intake is 1100 calories per day, the rate of metabolism may be sluggish. An immediate increase to 1900 calories per day might cause a fat gain because the body has adapted to a lower caloric intake and the sudden increase in calories would create a surplus of fat. The best approach would be to gradually increase the calories from 900 to 1900 over a few weeks to allow the metabolism to speed up and acclimatize appropriately.

Competency achievement: The student should be able to:

BI8.5: Summarize the nutritional importance of commonly used items of food including fruits and vegetables (micromolecules and their importance)

PE9.3: Explain the calorific value of common Indian foods

LAQ: Summarize the nutritional importance of commonly used items of food including fruits and vegetables (micromolecules and their importance)

Ans: There are seven major classes of nutrients: Carbohydrates, fats, dietary fibers, minerals, proteins, vitamins, and water. Carbohydrates are the most abundant dietary constituents and also the chief source of energy. Fats are a concentrated dietary source of energy, which contribute to about 20–50% of the energy requirement of the body. Although calories required for growth and maintenance of good health are obtained by eating carbohydrates, proteins, and fats, proteins are regarded as "body-building components" of food.

Minerals are useful in several ways as iron is required for hemoglobin synthesis; calcium for the normal formation of bones, teeth, and cellular processes; sodium and potassium to regulate homeostasis, iodine to produce thyroid hormones, etc.

Table 14.4 indicates the average nutritional values of various Indian food items.

Table 14.4: Average nutritional values of Indian foods

Food item	Protein, g	Carbohydrates, g	Lipids, g	Calories
1 cup rice	5	53	0.5	240
1 wheat chapati/roti	2	9–15	4.0	80
1 bajra roti	3	25	0.2	110
1 cup dal	14	10	4.0	200
1 medium size wheat bread	3	15	0.7	75
1 cup curd	14	10	4.0	150
1 egg	6	0.5	5.0	80
1 cup cow milk	8	12	2.5	100
1 cup buffalo milk	8	12	8.0	150
1 cup buttermilk	3	12	1.0	70
1 normal-size fish	10–15	1.0	2.5	200

Contd.

Table 14.4: Average nutritional values of Indian foods (Contd.)

Food item	Protein (g)	Carbohydrates (g)	Lipids (g)	Calories
1 normal-size banana	2	22–25	Nil	105
1 apple	0.5	2.5	0.3	95
1 plain dosa	2	17	3.0	120
1 idli	2	8	Nil	40

NOTE

1. There are average 150 calories in 1 cup of cooked vegetables. Calorie breakdown: 24% fat, 63% carbohydrates, 14% protein.
2. One cup of tea with a normal amount of sugar and milk offers 40 calories.
3. One cup of coffee with a normal amount of sugar and milk offers 50 calories.
4. Healthy normal BMI adult needs a daily average of 50–55 g protein, 275–350 g carbohydrates, 20–30 g lipids (saturated fats <10 g), and 2000–2200 calories with sufficient intake of micronutrients, and water.
5. One standard cup size = 250 ml.

Competency achievement: The student should be able to:

BI8.3: Provide dietary advice for optimal health in childhood and adulthood, in the diseases like diabetes mellitus, coronary artery disease, and in pregnancy

NUTRITIONAL NEEDS IN PREGNANCY AND LACTATION

LAQ: Write a note on nutritional needs in pregnancy and lactation.

Ans: During pregnancy and lactation nutritional needs increase to support the normal development of maternal tissues, related metabolic reactions, and the normal growth of the fetus and the newborn baby.

Maternal metabolism is adjusted through the effects of hormones that serve as mediators that redirect the nutrients to highly specialized maternal tissues specific to reproduction (i.e. placenta and mammary gland).

Additional energy intake: Since total energy expenditure does not change greatly and weight gain is minimum in the first trimester, additional energy intake is recommended only in the second and third trimesters. An additional average of 450 kcal is recommended during the second and third trimesters, respectively.

Additional protein intake: An additional 21 g of protein is required during pregnancy for the management of fetal, placental, and maternal tissues during the second and third trimesters. Women of reproductive age are expected to select diets containing average protein intakes of about 70 g/d.

Additional intake of vitamins and minerals: Plasma concentrations of many vitamins and minerals show a slow, steady decrease with the advance of gestation, which may be due to hemodilution. Deficiencies of vitamins and minerals may lead to fetal growth retardation and congenital anomalies.

Placental transport of vitamin A between mother and fetus is substantial, and recommended intake is increased by about 10%. Low blood levels of vitamin A are associated with intrauterine growth retardation. Adequate dietary supplementation with vitamin A is seen to reduce maternal mortality by 40%. Recommended dietary allowance of vitamin A for pregnant women: 800 µg RAE and for lactating women: 1300 µg RAE.

Vitamin D is responsive to increased maternal intake and it decreases in deficiency. Vitamin D is transported across the placenta to the fetus. Vitamin D deficiency during pregnancy is associated with disorders of calcium metabolism in both the mother and the infant. Calcium deficiency leads to neonatal hypocalcemia and tetany, infant hypoplasia of tooth enamel, and maternal osteomalacia. The average intake of vitamin D per day of 10 µg (400 IU)/d vitamin D in affected pregnant females was reported to decrease the incidence of neonatal hypocalcemia, osteomalacia, and tetany. Similarly, higher amounts of vitamin D up to 25 µg/d, were seen to be responsible for increased weight and normal growth of infants.

Compromised maternal folate intake is associated with several pregnancy complications including low birth weight, risk for spontaneous abortions, and neural tube defects. Folic acid 5 mg tablet per day with supplementation prevents both the occurrence and recurrence of neural tube defects and significantly reduces the incidence of low birth weight.

The total iron required during pregnancy is about 1040 mg, of which 200 mg is retained by the woman when blood volume decreases after delivery and 840 mg of iron is lost. About 300 mg of iron is transferred to the fetus and about 50–75 mg of iron is used for the formation of the placenta, about 450 mg of iron is used for the formation of new red blood cells, and about 200 mg of iron is lost in blood during delivery.

Total blood hemoglobin, serum iron, and serum ferritin concentrations decrease during pregnancy. However, transferrin levels increase in the last trimester of pregnancy, mainly to facilitate iron transfer to the fetus. About 3 mg/dl increase in the intestinal absorption of iron is useful in maintaining the daily iron requirement.

The anemic status of pregnant females is associated with infant mortality and premature delivery. The recommended iron intake during pregnancy is increased by 9 mg to 27 mg/d, to preserve maternal stores of iron and to prevent the development of iron deficiency, which is normally not obtained from foods. Hence, iron supplements are recommended to achieve the required intake of iron.

Maternal iodine deficiency may lead to fetal hypothyroidism which may lead to cretinism associated with severe mental retardation. Thyroid hormones are critical for normal brain development and maturation. Manifestation of other features of cretinism (deaf-mutism, short stature, and spasticity) depends on the stage of pregnancy and hypothyroidism. When hypothyroidism develops late in pregnancy, the neurological damage is not as severe as when it exists early in pregnancy. Cretinism is prevented by correcting maternal iodine deficiency before or during the first 3 months of pregnancy. The recommended iodine intake is 220 µg/d during pregnancy.

Nutritional needs during lactation: The recommended energy intake during the first 6 months of lactation is an additional 500 kcal under the assumption that 170 kcal/d will be mobilized from energy stores accumulated in pregnancy.

The nutritive demands of lactation are considerably greater than those of pregnancy. The recommended increased percentage intakes for energy and specific nutrients during lactation (compared to non-pregnant females) are as follows: 50% protein, 60% vitamin C, 85% vitamin A, 27% vitamin E, 90% iodine, 50% vitamin B_6, 25% other B-complex vitamins, and 50% zinc. Most of these recommended intakes are based on the knowledge of the amount of milk produced during lactation, its energy and nutrient contents, and the amounts of maternal energy and nutrient reserves.

Lactation is considered successful when the fully breast-fed infant grows well and maintains appropriate biochemical indexes of nutritional status. A supplement of vitamin D (10 µg/d) is recommended for women who avoid milk and other foods fortified with vitamin D. Similarly, a supplement of vitamin B_{12} (2.6 µg/d) is recommended for lactating women who are vegetarians.

LAQ: Provide dietary advice to pregnant and lactating females.

Ans: It is necessary to increase the nutritional needs during pregnancy and lactation for support of fetal and infant growth and development along with alterations in maternal tissues and metabolism. The following dietary plan is advised:

Additional energy intake: Approximately an additional 340 and 450 kcal are recommended during the second and third trimesters, respectively using the diet described below:

Additional protein intake: Select diets containing average protein intakes of about 50 g/d during the first trimester. The daily diet recommended to meet this requirement is as follows.

	Protein, g
Four chapati/roti:	4
1 cup rice:	5
2 cups dal:	28
1 egg:	6
1 cup cow milk:	8
Or	
1 cup buffalo milk:	8
1 cup curd:	25
1 cup = approximately	230 ml

An additional 20 g of protein is needed to cover the estimated fetal, placental, and maternal tissues during the second and third trimesters. Hence, the following food items are added to the daily diet: 1 egg, 1 cup milk, and 1 cup rice.

Females, that eat non-vegetarian foods, can eat 1 cup of meat three times a week. It provides 76 g per serving of chicken 1 cup three times a week. It provides 62 g of protein per serving.

Females having milk intolerance should eat 1/2 cup of peanuts (20 g protein) every day.

Female who does not eat egg is also recommended to eat ½ cup of peanuts every day. It provides 20 g per serving.

Women of reproductive age are expected to have the additional intake of vitamins and minerals as follows:

Since placental transport of vitamin A between mother and fetus is substantial, recommended intakes are increased by about 10%.

The main circulating form of vitamin D in plasma is 25-hydroxycholecalciferol. It is responsive to increased maternal intake and falls with maternal deficiency.

Folic acid supplementation prevents both the occurrence and recurrence of neural tube defects and significantly reduces the incidence of low birth weight.

Pregnant women should receive daily (RDA) vitamin A: Average 770 µg vitamin A (retinol): Per day: 1 egg (78 µg), 2 cup milk (70 µg), 1 carrot (300 µg), curd (80 µg) with supplements of vitamin A.

Vitamin D: Up to 600 IU vitamin D: Exposure to early morning sun for 30 minutes provides about 400 IU per day. Per day: 1 egg (44 IU), 2 cup milk (6 IU), curd (7 IU) with supplements of vitamin D.

Vitamin E: 22–30 mg. Daily consumption of an average of 3 spoons of cooking oil provides about 18 mg of vitamin E and 1 egg provides about 3 mg.

Thymine: 1.4 mg. Daily consumption of 2 cups of white rice provides about 1 mg thymine, or 2 cups of brown rice provides about 2 mg thymine.

Vitamin B_6: 1.9 mg. Daily consumption of 2 cups of milk provides about 0.2 mg, two bananas about 0.8 mg, one egg provides about 0.1 mg, and 1 standard piece of fish provides about 0.6 mg. Additional supplements in the form of tonic or tablets.

Folic acid: Average 400 µg. Daily consumption of 1 cup of cooked kidney beans provides about 130 µg, 1 cup of cooked lentils provides about 350 µg, 1 egg (22), 1 cup beat (130), and 1 cup of spinach (58). Additional supplements in the form of tonic or tablets.

Vitamin B_{12}: 4.0 µg: Daily consumption of 2 cup milk (2 µg), 1 cup curd (1 µg), 1 egg (0.6 µg). Additional supplements in the form of tonic or tablets.

Vitamin C: 80 mg. 1 cup of lemon juice provides 30 mg of vitamin C and 1 cup of orange juice provides about 50 mg of vitamin C.

Increase in 20% of other B-complex vitamins using supplements in the form of tonic or tablets.

Hemoglobin concentration declines during pregnancy along with serum iron, percentage saturation of transferrin, and serum ferritin. These decreases reflect hemodilution to a large extent. Maternal anemia is associated with perinatal maternal and infant mortality and premature delivery.

To preserve maternal stores and prevent the development of iron deficiency, the recommended iron intake during pregnancy is increased by 9 mg to a total of 27 mg/d. This level cannot normally be obtained from foods, and supplementation in the form of iron tablets with lemon juice or vitamin C tablets is required to achieve recommended intake.

Maternal iodine deficiency leading to fetal hypothyroidism results in cretinism, characterized by severe mental retardation. Thyroid hormones are critical for normal brain development and maturation. The recommended iodine intake is 220 µg/d during pregnancy. Daily consumption of 2 cup milk (170 µg), 1/4th spoon iodized salt in daily cooking (76 µg).

Nutritional needs during lactation: The recommended energy intake during the first 6 months of lactation is an additional 500 kcal under the assumption that 170 kcal/d will be mobilized from energy stores accumulated in pregnancy.

The nutritive demands of lactation are considerably greater than those of pregnancy. The recommended increased percentage intakes for energy and specific nutrients during lactation (compared to non-pregnant females) are as follows:

50% protein: Add to the diet further the following food items: 1 egg, 1 cup milk, 1 cup rice. Additional vitamin and mineral supplements are necessary in the form of tonic or tablets to meet the following requirements:

60% vitamin C: Increase intake of up to 120 mg

85% vitamin A: Increase intake of up to 1300 µg

27% vitamin E: Increase intake of up to 36 µg

90% iodine: Increase intake of up to 300 µg

50% vitamin B_6: Increase intake of up to 2.8 µg

25% other B-complex vitamins

50% zinc: Increase intake of up to 12 mg

Most of these recommended intakes are based on the knowledge of the amount of milk produced during lactation, its energy and nutrient contents and the amounts of maternal energy and nutrient reserves.

Lactation is viewed as successful when the fully breast-fed infant grows well and maintain appropriate biochemical indexes of nutritional status. A supplement of vitamin D (10 µg/d) is recommended for women who avoid milk and other foods fortified with vitamin D. Similarly, a supplement of vitamin B_{12} (2.6 µg/d) is recommended for lactating women who are complete vegetarians.

NUTRITIONAL NEED OF A NEWBORN

LAQ: Write a note on nutritional needs of a newborn.

Ans: For the nutritional needs of a newborn, it is necessary to determine total calories required from appropriate food. The calories needed for growth of a child will vary depending on the size, growth rate, and activity level of a child.

During first six months of age, nutritional requirements of a baby are met by breastfeeding by a healthy and adequately nourished mother. During first six months, most breastfed babies do not require additional vitamin or mineral supplementations. Vitamin D supplements are recommended for the babies who live in areas with very low sunshine or for babies who are kept covered due to cultural or religious traditions. In addition to basic nutritional needs of a baby, breastfeeding mothers also supply valuable antibodies to their babies through their breast milk. Following are dietary requirements of a newborn baby:

Energy requirement: 0–3 months: 116 kcal/kg/d. 3–12 months: 100 kcal/kg/d

Composition of the diet: The diet should contain 40% carbohydrates, 40–50% fat and 10% protein. The following are general guidelines for the diet given to a child:

- **0–6 months:** Breast milk (or cow protein-based formula) is used.
- **6–8 months:** Breast milk (or cow protein-based formula) and semi-solid food is used.
- **8–12 months:** Breast milk (or cow protein-based formula) and introduction of solid foods are used.

The following are the various advantages of breast feeding:

Breast feeding

- Supplies adequate nutrition for the first 6 months of life. It supplies adequate calories but need vitamin D supplementation, 400 IU/d, especially in dark skinned infants with inadequate sunlight exposure.
- Supplies adequate iron until 6 months of age. Later on the diet should be supplemented with iron fortified cereals. The requirement is 1 mg/kg/d.
- Helps to protect infant against respiratory and gastrointestinal infections and is useful to increase immunologic responses to vaccines.
- Decreases the incidence of breast, uterine, and ovarian cancer in breast feeding mothers. It may be useful to return to pre-pregnancy weight faster.

The following are general guidelines for providing diet to an infant:

1. High fat content of diet should continue throughout the first 2 years to ensure

adequate CNS growth and development. This may be accomplished with whole milk from 12–24 months.

2. If breast feeding curtailed before one year of age, iron fortified formula should be introduced.
3. Breast feeding should be avoided if mother is suffering from HIV, active tuberculosis, and if mother is receiving chemotherapy.
4. Cow's milk formula is recommended when breast milk is not available. It should be supplemented with necessary daily requirements of vitamins and iron (11–12 mg/liter). Cow's milk used in formula should be pasteurized, homogenized, or evaporated.
5. Unmodified cow's milk is not recommended for 1st year of life because it has high protein content, inadequate iron, low vitamin C, presents an increased solute load, and has decreased amounts of zinc.
6. There is no advantage for using soy based formulas over cow's protein formulas.
7. Goat milk is not recommended because of lack of folate and iron and it may not be adequately pasteurized.

BAQ: Enumerate four advantages of breast feeding to babies.

Ans: The following are various advantages of breast feeding:

1. Breast milk supplies adequate nutrition for the first 6 months of life. It supplies adequate calories but need Vitamin D supplementation, 400 IU/d, especially in dark skinned infants with inadequate sunlight exposure.
2. It supplies adequate iron until 6 months of age. Later on the diet should be supplemented with iron fortified cereals. The requirement is 1 mg/kg/d
3. Breast feeding helps to protect infant against respiratory and gastrointestinal infections and increase immunologic response to vaccines.
4. It decreases the incidence of breast, uterine, and ovarian cancer in breast feeding mothers. It may be useful to return to pre-pregnancy weight faster.

MALNUTRITION AND STARVATION

Competency achievement: The student should be able to:

PA12.2: Describe the pathogenesis of disorders caused by protein calorie malnutrition and starvation

PE9.1: Describe the age-related nutritional needs of infants, children, and adolescence including micronutrients and vitamins

BAQ: Define and describe malnutrition.

Ans: Malnutrition is the condition that results from taking an unbalanced diet in which there is deficiency of essential nutrients. The World Health Organization cites malnutrition as the greatest single threat to the world's public health. Improving nutrition is widely regarded as the most effective form of getting rid of malnutrition.

Malnutrition increases the risk of infection and infectious disease. It is a major risk factor in the onset of active tuberculosis. In communities or areas that lack access to safe drinking water, malnutrition adds to health risks, which may lead to a critical health-related problem. Low energy levels and impaired function of the brain due to malnutrition, the patients are unable to perform the normal activities in order to gain general quality life.

SAQ: What is cachexia? Mention two diseases that cause cachexia.

Ans: Cachexia or wasting syndrome is loss of weight, muscle atrophy, fatigue, weakness, and significant loss of appetite in someone who is not actively trying to lose weight. The secretion of cytokines in response to infection and cancer increases the catabolism of tissue protein, that lead to cachexia. Cachexia is observed in patients suffering from AIDS and tuberculosis.

BAQ: Write a brief note on kwashiorkor.

Ans: Kwashiorkor is an acute form of childhood protein-energy malnutrition. Susceptibility to kwashiorkor increases after 18 months of age. It is characterized by edema, irritability, anorexia, ulcerating dermatoses and an enlarged liver with fatty infiltrates. The presence of edema caused by poor nutrition indicates kwashiorkor, and it is associated with a significant decreased concentration of plasma proteins. Deficiency of micronutrient and antioxidants are also contributory factors. An infection may precipitate kwashiorkor. Cases of kwashiorkor in the developed world are rare.

BAQ: Write a note on marasmus.

Ans: Marasmus is a form of severe protein-energy malnutrition characterized by energy deficiency. It can occurs mainly in the babies less than one-year-old. Marasmus is generally known as the gradual wasting away of the body due to severe malnutrition or inadequate absorption of food. It is a form of severe protein deficiency and is one of the forms of protein-energy malfunction. Marasmus is characterised by total muscle loss, anemia, dehydration, dry skin, brittle hair, and anemia. Edema is not observed.

Marasmus is caused by a severe deficiency of nearly all nutrients, especially protein and carbohydrates, leading to prolonged negative energy balance. In marasmus, the fat reserves of body are exhausted with loss of protein from the liver and tissues. To maintain a supply of glucose, amino acids from catabolized proteins are used by gluconeogenesis. Due to reduced synthesis of proteins, there is impaired immune response and increased risk from infections.

Impairment in the cell proliferation of intestinal mucosa leads to poor absorption of nutrients. A child with marasmus looks emaciated and body weight of affected child may be reduced to less than 80% of the average weight that corresponds to the height.

Marasmus can be distinguished from kwashiorkor by the lack of edema.

BAQ: Describe marasmus under the following heads:

1. Biochemical basis
2. Clinical features
3. Diagnostic laboratory tests
4. Treatment

Ans:

1. **Biochemical basis:** Marasmus is caused by a severe deficiency of nearly all nutrients, especially protein and carbohydrates, leading to prolonged negative energy balance.
2. **Clinical features:** Significant loss of weight. Significantly decreased body mass index, 16< BMI of the patient. Dry skin and eyes. Edema is not observed. Diarrhea, low blood pressure.
3. **Diagnostic laboratory test results:** Serum total proteins < 5.0 g/dl, Serum albumin< 2.5 g/dl. Hemoglobin <10 g/dl.
4. **Treatment:** First it is necessary to treat underlying disease. Then, marasmus is treated through three stages: Rehydration, stabilization and nutritional rehabilitation and follow-up.

Rehydration: The patient is treated with rehydrating solution containing electrolytes, glucose, amino acids, vitamins, and minerals by mouth or by vein.

Stabilization: Liquid diet is provided with approximately 80–100 kilocalories per kilogram per day (kcal/kg/d) spread over 8–12 meals per day for three to seven days.

Nutritional rehabilitation and follow-up: Locally available, nutrient-dense foods containing multi-vitamins and minerals to improve nutritional status and weight, till BMR rises >18. Patient should stick to balanced diet later on to maintain normal BMR.

Competency achievement: The student should be able to:

BI8.2: Describe types and causes of protein energy malnutrition and its effects

Protein-Energy Malnutrition

LAQ: Define and describe protein-energy malnutrition and its effects.

Ans: Protein-energy malnutrition (or protein-calorie malnutrition) refers to a form of malnutrition where there is inadequate protein intake. Food intake less than expenditure leads to various clinical conditions such as emaciation, wasting, marasmus and kwashiorkor. Children are affected the most by protein-energy malnutrition; if they have less protein intake. Patients with HIV infection, chronic diseases and advanced cancer also suffer from cachexia and are frequently undernourished. Severe nourishment deficiency is associated with increased mortality.

Competency achievement: The student should be able to:

PE10.1: Define and describe the etiopathogenesis, classify including WHO classification, clinical features, complications and management of severe acute nourishment (SAM) and moderate acute nourishment (MAM)

PE10.2: Outline the clinical approach to a child with severe acute nourishment (SAM) and moderate acute nourishment (MAM)

PE10.3: Perform assessment of a patient with severe acute nourishment (SAM) and moderate acute nourishment (MAM), diagnosis, classification and planning management including hospital and community-based intervention, rehabilitation and prevention

SEVERE ACUTE MALNUTRITION (SAM) AND MODERATE ACUTE MALNUTRITION (MAM)

LAQ: Define, classify and describe the etiopathogenesis, clinical features, complications and management of severe acute nourishment (SAM) and moderate acute nourishment (MAM).

Ans: The currently accepted definitions of malnutrition defined by WHO are as follows:

1. **Moderate acute malnutrition (MAM)**, defined as weight-for-height z-score (WHZ) between –2 and –3 or mid-upper arm circumference (MUAC) between 115 millimeters and <125 millimeters (WHO 2012).
2. **Severe acute malnutrition (SAM)**, defined as WHZ < –3 or MUAC < 115 millimeters, or the presence of bilateral pitting edema, or both (WHO 2013)
3. **Global acute malnutrition (GAM)** refers to MAM and SAM together. It is used as a measurement of nutritional status at a population level and as an indicator of the severity of an emergency situation (Global Nutrition Cluster (GNC) 2014).

Each year, a large number of children around the world die before the age of five years. The leading causes of deaths among the children are prematurity, malnutrition and respiratory disease like pneumonia, which are responsible for about 16 percent of all deaths in this age group. Degrees of malnutrition are associated with increased risk of mortality and increased risk of death due to diarrhea, pneumonia, and measles.

Acute malnutrition results from reductions in food intake or diet quality. It is often combined with pathological causes such as protein-energy malnutrition, wasting, kwashiorkor, and marasmus.

Marasmus and kwashiorkor are common terms historically used to differentiate between types of SAM. Marasmus refers to children who are very thin for their height (means, they meet the WHZ or MUAC cut off) but do not have edema. Kwashiorkor refers to signs of malnutrition with edema. The most recent WHO terminology for SAM has replaced these terms.

Treatment and Nutritional Support

The following are treatment and nutritional support measures for children suffering from SAM and MAM:

1. First it is necessary to treat underlying disease. Child with profuse watery diarrhea or suspected cholera, should be rehydrated with full-strength WHO low-osmolarity oral rehydration solution.
2. Children who are severely dehydrated or with signs of shock should be rehydrated intravenously, using half-strength Darrow's solution with 5 percent dextrose, Ringer's lactate solution with 5 percent dextrose, or with 0.45 percent saline with 5 percent dextrose (WHO 2013).
3. Infections should be treated routinely upon admission by provision of a broad-spectrum antibiotic, and measles vaccination should be given for unimmunized children older than age six months.
4. Then, the patient is treated through three stages: Rehydration, stabilization and nutritional rehabilitation and follow-up:
   - *Rehydration:* The patient is treated with rehydrating solution containing electrolytes, glucose, amino acids, vitamins, and minerals by mouth or by vein.
   - *Stabilization:* Liquid diet is provided with approximately 80–100 kilocalories per kilogram per day (kcal/kg/d) spread over 8–12 meals per day for three to seven days.
   - *Nutritional rehabilitation and follow-up:* Locally available, nutrient-dense foods containing multi-vitamins and minerals to improve nutritional status and weight, till BMR rises >18.
5. Ready-to-use-foods (RUFs) are useful in specific areas, which contain specially formulated biscuits, bars, or pastes that provide varying ranges of high-quality protein, energy, and micronutrients. These products are more nutrient dense than home foods and do not require preparation. Patient should be given balanced diet later on to maintain normal BMR.

LAQ: Describe the strategies for prevention of acute nourishment (SAM) and moderate acute nourishment (MAM).

Ans: For the prevention of SAM and MAM, following are the strategic points:

1. The promotion of appropriate breast-feeding and complementary feeding practices for children.
2. Easy access of children and parents to appropriate healthcare for the prevention and treatment of disease, and improved sanitation and hygiene practices.
3. Free supply of multiple-micronutrient powders, small-quantity lipid nutrient supplements (LNSs) and single-nutrient supplements to augment the nutritional content of the home diet.
4. Follow-up of the 2012 WHO technical note on supplementary foods for managing MAM in children ages 6–59 months used for providing locally available, nutrient-dense foods to improve nutritional status and prevent SAM (WHO 2012).
5. Follow-up on WHO (2012) suggestions: An energy intake of 25 kcal/kg/d in addition to the standard nutrient requirements of a normal nourished child. This would support a reasonable rate of weight gain without promoting obesity.
6. Adopting the 2013 WHO guidelines (WHO 2013), which recommend that children should be enrolled and discharged from treatment using the same mode of classification.

   Children who were admitted based on MUAC should be discharged once their MUAC is ≥125 millimeters for at least two weeks or their WHZ is ≥–2 for at least two weeks.

   Children who were admitted based on their edema should be discharged based on the measurement routinely used in the program. Once discharged, the children should be followed up periodically to avoid relapse.

7. Adopting the 2013 WHO guidelines (WHO 2013), which include the following additional updates: Children who are not treated with fortified therapeutic foods should receive a high dose of vitamin A on admission. Children who receive therapeutic food do not need the high dose of vitamin A.
8. Ready to use therapeutic foods (RUTFs) should be given to children regardless of whether they have diarrhea.
9. Follow-up on the community based management of severe acute malnutrition (CMSM) model guidelines, which state that treatment programs for SAM should achieve a case fatality rate (CFR) of less than 10 percent, a recovery rate greater than 75 percent, and a defaulter rate of less than 15 percent.

LAQ: Describe the WHO's programme for the "In patient treatment" of acute nourishment (SAM) and moderate acute nourishment (MAM).

Ans: The following are steps described by the WHO for the "inpatient treatment" of acute nourishment (SAM) and moderate acute nourishment (MAM):

1. Initial treatment given to take care of hypoglycemia, dehydration, infections, hypothermia, and electrolyte imbalances, and micronutrient deficiencies are corrected.
2. Rehabilitation measures require correction of electrolyte imbalances and micronutrient and iron deficiencies using feeding formulae **(F-75 and F-100)** suggested by WHO. Feeding is increased to stimulate growth, and children are prepared for discharge.

   Formula 75 (F-75) contains 75 kcal and 0.9 g protein per 100 ml. Formula 100 (F-100) contains 100 kcal and 2.9 g protein per 100 ml. Once the child is stabilized on F-75, F-100 is used to rebuild wasted tissues.
3. Follow-up measures suggest increased feeding to recover weight loss.
4. Micronutrient deficiencies should be treated by giving vitamin A (200,000 international units [IU] for children older than age 12 months, 100,000 IU for children ages 6–12 months, and 50,000 IU for children ages 0–5 months), coupled with daily multivitamin, folic acid, zinc, and copper supplementation for at least two weeks. Iron supplementation should be given once children have begun gaining weight.
5. During the rehabilitation phase, F-75 should be replaced with F-100 in the same amounts for 48 hours. Children could be transitioned from F-75 to ready to use therapeutic food (RUTF) according to the updated WHO guidelines (WHO 2013).
6. Respiratory rate and pulse rates of the child should be monitored closely. After transition to F-100, children should receive feedings consisting of 100–200 kcal/kg/d and 4–6 g protein/kg/d at least every four hours. Breastfeeding should continue to be encouraged.
7. After recovery, parents should be taught to feed children frequently with energy and nutrient dense foods and to continue to stimulate sensorial and emotional development of children.
8. Parents should be requested to bring children back for regular follow-up checks. Vitamin A supplementation and booster immunizations should be provided.
9. In addition to increased susceptibility to infections, children with SAM are more likely to have more severe illnesses and higher mortality rates than nonwasted children. The common infections include, acute respiratory infection, diarrhea, tuberculosis, meningitis, anemia, HIV, bacteremia, and sepsis. Treatment for malnourished children and their mothers with concurrent infections should follow the WHO guidelines.

BAQ: Enumerate five main risk factors for MAM and SAM.

Ans: Based on scientific literature investigating the relationships among specific individual, household, and environmental factors and the development of acute malnutrition in children, the following are significant risk factors for MAM and SAM:

1. Lack of parental education
2. Big family size
3. Incomplete vaccination
4. Poverty
5. Inadequate dietary intake

Competency achievement: The student should be able to:

BI8.4: Describe the causes (including dietary habits), effects and health risks associated with being overweight/obesity

HEALTH RISKS ASSOCIATED WITH OBESITY

LAQ: Describe the causes (including dietary habits), effects and health risks associated with obesity.

Ans: Obesity is defined as excessive accumulation of fat in the case on an individual; due to which the body mass index (BMI) increases above 30.

The World Health Organization has classified obesity as a disease. Several interventional studies conducted all over the world indicate that significant increase in obesity is on the rise in the populations of various countries in the world.

According to the fifth round of the National Family Health Services (NFHS) of Government of India, conducted from the years 2019–2021, about 6.4 per cent of women and 4.0 per cent of men aged 15–49 are obese. It has been estimated that worldwide large number of children under the age of 5 are obese, and one in 10 children is overweight. India has the second-highest number of obese children globally, behind China.

Obesity leads to several health problems such as hypertension, stroke, hypercholesterolemia leading to heart diseases, and insulin resistance leading to type II diabetes and related decrease in the functions of the immune system. Obese children are prone to insulin resistance and related Type 2 diabetes mellitus and metabolic syndrome at very early age.

The combination of following main factors is responsible for the observed high obesity rates:

1. Changes in diet and lifestyle
2. The adoption of sedentary lifestyles
3. The socioeconomic and cultural factors.
4. The availability of cheap, processed foods

The state of obesity clearly contributes to insulin resistance, which in turn can cause type 2 diabetes. Virtually all obese and most type 2 diabetic individuals have marked insulin resistance.

Due to obesity resistance is also created to the functions of the hormone leptin, which is an appetite suppressant. Leptin is produced by specific gene in fat cells. Both insulin and leptin normally function as satiety signals to the hypothalamus in the brain. However, insulin and leptin resistance may reduce this signal and therefore facilitate continued overfeeding in spite of large body fat stores. Similarly, reduced leptin signalling to the brain also may reduce normal effect of leptin to maintain an appropriately high metabolic rate.

According to World Cancer Research Fund and American Institute for Cancer Research, reports that there is significant relation between lifestyle, including food consumption and cancer prevention. The same report recommends eating mostly foods of plant origin and aiming to meet nutritional needs through diet alone. It is also necessary to limit consumption of energy dense foods, red meat, alcoholic drinks, salt and avoiding sugary soft drinks and processed meat to prevent obesity.

SAQ: Define and enumerate the main causes of obesity.

Ans: Definition: Obesity is defined as excessive accumulation of fat in the case on an individual; due to which the body mass index (BMI) increases above 30.

The combination of the following main factors is responsible for the observed high obesity rates:

1. Changes in diet and lifestyle
2. The adoption of sedentary lifestyles
3. The socioeconomic and cultural factors.
4. The availability of cheap, processed foods

Competency achievement: The student should be able to:

PE11.1: Describe the common etiology, clinical features and management of obesity in children

Childhood Obesity in India

LAQ: Describe the common etiology, clinical features and management of obesity in children.

Ans: It has been estimated that worldwide a large number of children under the age of 5 are obese, and one in 10 children is overweight. India has the second-highest number of obese children globally, behind China. Overweight is defined as excess body weight relative to height. The obesity mainly occurs due to energy imbalances between calories consumed and calories exhausted and also due to excessive calorie intake with lack of physical activity. Genetic, epigenetic and changed lifestyles are the main reasons that are responsible for the increase in childhood obesity.

Obesity in children may lead to premature insulin resistance leading to early development of Type 2 diabetes mellitus and metabolic syndrome if obesity is not treated well.

Following are the main factors responsible for childhood obesity:

- Lack of physical activity
- Overindulgence in use of mobile phone and games and TV
- Lack of outdoor games and exercises
- Excessive food intake
- Lifestyle related factors
- Lack of knowledge about nutrition in parents, particularly mother
- Mother or father suffering from Type 1 or Type 2 diabetes mellitus
- BMI of mother or father > 25
- Limited availability of open spaces and parks needed for exercise and games
- Fast-food eating habits

OBESITY PREVENTIVE STRATEGIES

Obesity preventive strategies include:

1. Primary prevention of gaining excess weight or obesity and
2. Secondary prevention of weight regains following weight loss, and prevention of further weight increases.

Strategies to reduce childhood obesity: Following various strategies could be adopted to reduce childhood obesity:

The rate of childhood obesity could be reduced by:

1. Setting up policies to determine average normal calorie intake and BMR of children in the age groups 1–4, 5–10, and 10–15. Once normal BMR values and average calorie intake are determined in a particular population, it is possible to find out number of obese children and their growing trait by comparing with normal BMI values.
2. Performing clinical laboratory tests among children at early age to diagnose any genetic disease or any other disease related to obesity.
3. Decreasing the various factors that may give rise to obesity.
4. Introducing prevention of obesity related education at school level.
5. Increasing an awareness in the community and using preventive actions towards managing obesity risks.
6. Introducing free health programs at district or municipal hospital level for monitoring periodically of nutritional and obesity status of children and their parents.
7. Creating a database for childhood obesity at various regions of India.

8. Initiating community-based research to document burden of obesity and associated risk factors and various measures to monitor obesity trend from time to time.
9. Introducing nutrition and physical advice by the ruling governments through audio-visual media.
10. Encouraging organization and participation of children in health walks and healthy food festivals.

DIETARY PLANS IN DISEASE

LAQ: Advise a dietary plan to a person suffering from diabetes mellitus.

Ans: Normal glycemic control is very important for a diabetic person. Uncontrolled diabetes mellitus may lead to diabetic complications such as, neuropathy, nephropathy, retinopathy and atherosclerosis. Normal glycemic control could be maintained by a planned diet, which helps to prevent fluctuations in blood glucose levels in 24 hours. The following instructions should be used during 24 hours and then patient should follow the prescribed diet:

1. Avoid fasting on any particular day.
2. Maintain normal glycemic control by regular use of the drugs prescribed by the physician.
3. Daily walk for about 30–45 minutes
4. Undergo occasional blood tests for blood sugar, glycosylated hemoglobin and lipid profile.
5. Avoid table sugar, cakes, pastries, sweets, excess cheese, fast food and soft drinks.
6. Maintain daily normal water intake (average 6–7 glasses of safe drinking water).
7. Include more non-starchy vegetables, such as spinach, green beans, broccoli, in the diet.
8. Use whole foods (unprocessed and in the natural form) instead of highly processed foods.
9. Maintain normal BMI, by regular checking of weight.

Diet for Diabetic Persons

Breakfast (8–9 AM)

- A cup of tea or coffee or milk
- A bowl of oats porridge or one boiled egg (when serum cholesterol is normal)
- Or wheat flakes with milk and one fist dry fruits
- Or vegetable-moong dal preparation made with at least 2–3 vegetables
- Or A bowl of Upma or Pohe
- Or 2 chapatis with 1 bowl of cooked vegetables (any green leafy vegetable such as spinach, radish, methi etc.) and a cup of curd
- Or 2 slices of whole wheat bread + egg white omelette with a bowl of cooked vegetables

Mid-Morning (11–11.30 AM)

- A cup of green tea with a handful of roasted gram
- OR one whole fruit (apple/pear/orange/2–3 slices of papaya/guava)

Lunch (1–1.30 PM)

- 1 bowl of salad + 2 chapatis with 1 big bowl of cooked vegetable + 1 bowl of dal/sprouts/curd/buttermilk/or 2–3 pieces of chicken or fish or one boiled egg (whole egg when serum cholesterol is normal, otherwise only egg white)
- Or 1 big bowl of mung dal khichdi with curd+ 2 chapatis with one bowl of cooked vegetables and one fist dry fruits

Evening Snacks (4–5 PM)

- 1 whole fruit (apple/pear/orange/2–3 slices of papaya/guava) and 1 fist gram (boiled or roasted)
- Or mixed bhel (cucumber, tomato, green peas, onion, coriander)
- Or khakhra (2–3)
- Or vegetable sandwich (avoid butter, cheese and mayonnaise)

Dinner (8–9 PM)

One bowl of mung dal with curd + two chapatis and one bowl of cooked vegetables

LAQ: Advise a dietary plan to a person suffering from coronary artery disease (CAD).

Ans: Coronary artery disease is caused by atherosclerosis; means narrowing of arteries that supply blood to the coronary arteries. Apart from the genetic reasons (primary conditions), due to certain diseases such as diabetes mellitus, renal disease, etc., (secondary conditions), and diet rich in lipids may increase rate of atherosclerosis.

The main aim of prescribed diet to a CAD patient is to reduce the risk of cardiovascular disease by encouraging the patient to eat diet low in lipids.

The following instructions should be used during 24 hours and then patient should follow the prescribed diet:

1. Use prescribed drugs of the physician regularly and punctually, (drugs related to blood thinning, hypolipidemic and for the normal control of blood pressure). If diabetic, maintain normal glycemic control by regular use of the drugs prescribed by the physician.
2. Daily walk for about 30–45 minutes
3. Undergo occasional blood tests for blood sugar, glycosylated hemoglobin and lipid profile tests
4. Avoid egg yellow, liver, sweets, cakes, pastries, festival sweets, cheese, fast food and soft drinks.
5. Maintain daily normal water intake (average 6–7 glasses of safe drinking water).
6. Include more non-starchy vegetables, such as spinach, green beans, broccoli, in the diet.
7. Use whole foods (unprocessed, in the natural form) instead of highly processed foods.
8. Maintain normal BMI by regular checking of weight.

DIET FOR PATIENTS SUFFERING FROM CORONARY ARTERY DISEASE

Breakfast (8–9 AM)

- A cup of tea, one glass carrot juice
- And a bowl of oats with fat free milk
- Or wheat flakes with fat free milk and one fist dry fruits
- Or vegetable—moong dal preparation made with at least 2–3 vegetables
- Or a bowl of upma or Pohe
- Or 2 chapatis with 1 bowl of cooked vegetables (any green leafy vegetable such as spinach, radish, methi, etc.) and a cup of curd
- Or 2 slices of whole wheat bread + one egg white with a bowl of cooked vegetables

Mid-Morning (11–11.30 AM)

A cup of green tea with a handful of roasted gram OR one whole fruit (apple/pear/orange/2–3 slices of papaya/guava)

Lunch (1–1.30 PM)

- 1 bowl of salad + 2 chapatis (without ghee) with 1 big bowl of cooked vegetable + 1 bowl of dal/sprouts/curd/buttermilk/ or 2–3 pieces of chicken or fish (non-oily), or cooked one egg white.
- Or 1 big bowl of mung dal khichdi with curd+ 2 chapatis (without ghee) with one bowl of cooked vegetables and one fist dry fruits.

Evening Snacks (4–5 PM)

- 1 whole fruit (apple/pear/orange/2–3 slices of papaya/guava) and 1 fist gram (boiled or roasted)
- Or mixed bhel (cucumber, tomato, green peas, onion, coriander)
- Or khakhra (2–3)
- Or vegetable sandwich (avoid butter, cheese and mayonnaise)

Dinner (8–9 PM)

One bowl of moong dal with curd + Two chapatis and one bowl of cooked vegetables.

LAQ: Advise a dietary plan to a immunocompromised person.

Ans: Immunocompromised persons have weak immune system due to a specific disease or clinical condition from which they suffer. They can easily succumb to infectious diseases. A large number of immunocompromised persons died during COVID-19 pandemic during the years 2019–2023. However, proper diet could be useful to maintain satisfactory health status. The following is the diet prescribed for immunocompromised persons:

DIET FOR IMMUNOCOMPROMISED PERSONS

Breakfast (8–9 AM)

- A cup of tea or coffee or milk
- Two slices of brown bread and one boiled egg
- Or Wheat flakes with milk
- Or one plate of pohe or upma or sheera or two idlis
- Or 2 chapatis with 1 bowl of cooked vegetables (any green leafy vegetable such as spinach, radish, methi etc.) and a cup of curd

Mid-Morning (11–11.30 AM)

- A cup of vegetable or chicken soup and one whole fruit (apple/pear/orange/2–3 moon slices of papaya/guava)

Lunch (1–1.30 PM)

- 1 bowl of salad + 2 chapatis with 1 big bowl of vegetable + 1 bowl of dal/sprouts/curd/buttermilk/or 2–3 pieces of chicken or fish or one boiled egg and one bowl of rice
- Or 1 big bowl of moong dal khichdi with curd+ 2 chapatis with one bowl of cooked vegetable.

Evening Snacks (4–5 PM)

Tea or coffee and 1 whole fruit (banana/apple/pear/orange/2–3 slides of papaya/guava) and two pieces of brown bread or khakhra.

Dinner (8–9 PM)

- One big bowl of vegetable dal khichdi with curd + two chapatis and one bowl of rice
- Avoid excess of table sugar, sweets, cakes, pastries, festival sweets.
- All meats should be thoroughly cooked, including chicken or fish and eggs.
- Fresh raw fruits and vegetables must be washed thoroughly, under running water.
- Avoid eating excess cheese.
- Use only pasteurized milk and milk products, including cheese and yogurt.
- Avoid fast food and cold drinks.
- Daily normal water intake about 6–7 glasses.

Competency achievement: The student should be able to:

IM23.4: Enumerate the indications for enteral and parental nutrition in critically ill patients

IM23.2: Discuss and describe the causes and consequences of protein caloric malnutrition in the hospital

CRITICAL ILLNESS AND NUTRITIONAL SUPPORT

LAQ: Enumerate the indications for enteral and parental nutrition in critically ill patients.

Ans: Critical illness is associated with increase in catabolic rate of biochemical reactions due to increased stress and systemic inflammatory responses. The following factors contribute to stress: Multiorgan failure, increased infectious morbidity and prolonged hospitalization.

Various metabolic changes in critically ill patients tend to increase the risk of malnutrition. Decrease in total calories and protein intake are responsible for the deterioration of clinical condition of a critically ill patient. Increase in sepsis, rise in inflammatory biomarkers, and metabolic imbalance may result in shock, multiple organ failure, and mortality.

It is necessary to provide adequate nutrition in order to decrease metabolic response to stress and modulate immune responses favourably. Nutritional support in critically ill patients prevents further metabolic deterioration and loss of lean body mass.

Recovery in the Intensive Care Unit (ICU) is significantly effective by appropriate timing of initiation of proper feeding route and amount and type of nutrition provided to the critically ill patient. Initiating feeding within 24–48 hours of critical illness is necessary as an early nutrition intervention.

Use of Enteral or Parenteral Feeding Routes

It is necessary to use enteral or parenteral feeding routes on the basis of the assessment of hemodynamic status and gastrointestinal functioning. This feeding approach will prevent the risks associated with the inappropriate feeding techniques.

Enteral feeding (EN) means nutrition taken through the mouth or through a tube that goes directly to the stomach or small intestine.

Parenteral nutrition (PN) is intravenous administration of nutrition, which may include protein, carbohydrate, fat, minerals and electrolytes, vitamins and other trace elements for patients who cannot eat or absorb enough food through tube feeding formula or by mouth to maintain good nutrition status.

Early enteral nutrition (EEN) in critically ill patient is found to be associated with many benefits. EEN reduces risk of complications.

Following supplements are recommended for the eternal feeding (EN):

1. Commercially prepared products such as organic food mixtures, usually available as drinks is used for EN. Two food supplements per day are usually recommended. Organic food mixtures are available in vegetable mix and vegetable and nonvegetable combinations. Conventional EN products contain processed ingredients such as corn syrup, casein, sucrose, maltose, whey and soy proteins and oil, corn oil, and specific quantity of fiber.
2. The blenderized liquid diet in safe drinking water is used as a step between a full liquid diet and a regular diet. This diet consists of fluids and foods blenderized to a liquid form.

Nutrition Feeding Guidelines

Following are recommended nutrition feeding guidelines:

1. All the critically ill patients should undergo nutrition assessment, on admission and further evaluation by body profile tests.
2. Observation of signs of malnutrition (e.g. cachexia, edema, muscle atrophy, BMI <18 kg/m^2).
3. EN should be started early, preferably within first 24–48 hours.
4. Drug–nutrient interactions must be assessed daily. A multidisciplinary team including nutritionists should assess probable drug–nutrient interactions on daily basis.
5. In case the nutrition requirement is not met adequately with EN even after 7 days of ICU admission, then usage of parenteral nutrition (PN) may be considered.
6. Electrolytes (normal saline) should be strictly monitored in the patient on nutrition therapy.
7. PN to be considered if even 50–60% of nutrition targets are not met adequately within 72 hours of oral nutrition support.

Competency achievement: The student should be able to:

IM23.1: Discuss and describe the methods of nutritional assessment in an adult and calculation of caloric requirements during illness

IM24.22: Describe and discuss the aetiopathogenesis, clinical presentation, complications, assessment and management of nutritional disorders in the elderly

NUTRITIONAL DISORDERS IN THE ELDERLY

LAQ: Describe and discuss the aetiopathogenesis, clinical presentation, complications, assessment and management of nutritional disorders in the elderly.

Ans: Aging is a natural process. Aging is defined as the time-related deterioration of the physiological functions necessary for survival and fertility. Aging leads to the progression of disabilities and diseases that affect normal body functions:

1. Aging is a major risk factor for neurodegenerative diseases.
2. Arthritis is a major disease that limits the activities of older adults, and osteoarthritis is the most common form of the disease. The prevalence of osteoarthritis increases directly with age.
3. Aging affects oral tissues in addition to other parts of the human body, and oral health. Oral diseases can have a profound impact on general health.
4. Some hearing and vision loss and decline in immune functions are a part of normal aging.
5. Obesity in elderly persons is a risk factor for other diseases such as Type 2 diabetes mellitus, cardiovascular disease and hypertension.
6. Undernutrition is common in older people and it has serious adverse effects. Weight loss and low body weight are significant markers. Protein energy malnutrition may be the direct result of poor diet. It may develop indirectly when other illnesses increase nutritional requirements beyond usual needs.
7. The following diseases suffered by older persons are the result of dietary changes related to lifestyle changes and immunocompromised state: Cancer of the colon, pancreas and prostate, diabetes, and osteoporosis.
8. Significant deficiencies in vitamin D and B_{12} and micronutrients.

If nutritional disorders are identified and managed appropriately, the health of many elderly individuals can be significantly improved.

It is necessary that the daily intake of foods in the case of elderly contains appropriate amounts of proteins, carbohydrates, lipids, vitamins, other micronutrients, and food supplements.

Assessment of nutritional disabilities and diseases in the older adults:

Routine check up from the physician and complete hemogram test with routine examination of urine, feces occult blood test, blood glucose, lipid profile and various organ function tests give good indication of nutritional deficiencies and in general health status of an elderly person.

The following diet is suggested for an elderly person:

Diet for elderly individuals

Breakfast (8–9 AM)

- One glass of carrot and apple mixed juice
- A cup of tea or coffee or milk
- Two slices of brown bread; Or wheat flakes with milk Or one plate of pohe or upma or sheera or two idlis
- One vitamin B-complex capsule

Mid-Morning (11–11.30 AM)

A cup of cooked vegetables or chicken soup and one whole fruit (apple/pear/orange/2–3 slices of papaya/guava) and one cup of milk with 3 spoons of protein supplements

Lunch (1–1.30 PM)

- 1 bowl of salad + 2 chapatis with 1 big bowl of cooked vegetable + 1 bowl of dal/sprouts/curd/buttermilk/or 1–2 pieces of chicken or fish and one bowl of rice
- Or 1 big bowl of moong dal khichdi with curd+ 2 chapatis with one bowl of cooked vegetables
- Calcium tablet: 500 mg (first 3 weeks of a month only)

Evening Snack (4–5 PM)

Tea or coffee and 1 whole fruit (banana/apple/pear/orange/2–3 slides of papaya/guava) and two pieces of brown bread or khakhra.

Dinner (8–9 PM)

Two chapatis and one bowl of cooked vegetables.

NOTE

1. 5–6 gm of protein supplements in the form of whey, casein or peanut hydrolysate respectively are recommended per day by considering tolerance and allergic reactions of the elderly person to any one or all of the forms of proteins mentioned above.
2. It is necessary to avoid potato chips, French fries, and other "junk" foods, Vegetables cooked in butter, cheese, or cream sauces, fried foods, whole milk (if milk intolerance is there), bacon, sausages, egg yolks, cheese cake, pastries, doughnuts, ice creams, butter and margarine.
3. All meats should be thoroughly cooked, including chicken or fish. No raw or undercooked vegetables or meats.
4. Fresh raw fruits and vegetables must be washed properly under running water before eating them.

SAQ: Enumerate diseases suffered by older persons due to malnutrition.

Ans: The following diseases suffered by older persons are the result of dietary changes related to lifestyle changes and immunocompromised state: Increased blood pressure, decreased glucose tolerance, increased blood lipids, degenerative diseases such as cardiovascular and cerebrovascular disease, and cancer of the colon, pancreas and prostate, diabetes, and osteoporosis.

Competency achievement: The student should be able to:
SU1.1: Describe basic concept of homeostasis, enumerate the metabolic changes in injury and their mediators

SAQ: Describe the basic concept of homeostasis.

Ans: Refer to Ch 1, p6

Competency achievement: The student should be able to:
SU1.2: Describe the factors that affect the metabolic changes in injury and their mediators

BAQ: Describe the factors that affect the metabolic changes in injury and their mediators.

Ans: Stress response caused by trauma includes endocrine, metabolic and immunological changes. Stress hormones such as epinephrin, norepinephrine and cytokines are responsible for various biochemical reactions. The characteristic responses that occur in trauma patients are as follows:

1. Body fat and protein catabolism to meet energy requirements of body cells.
2. Preservation of body fluids and electrolytes to cope up with metabolic changes caused by hypermetabolism.
3. The oxygen and energy requirements increase in proportion to the severity of trauma.
4. Alterations in amino acid, lipid, and carbohydrate metabolism for recovery from tissue injury.
5. Reduction of the normal anabolic effect of insulin by the development of insulin resistance.
6. Severe trauma, burns and sepsis result in increased protein degradation.

NUTRITIONAL SUPPORT FOR THE PATIENT IN TRAUMA

The details of metabolic response to trauma should be known in managing trauma cases and the patient should be treated accordingly. The following are the important points to note:

1. The main aim of administration of glucose-saline to fasting surgical patients is to reduce proteolysis and to prevent loss of muscle mass.
2. Diet containing free fatty acids are primary sources of energy after trauma. Triglycerides meet nearly 60 to 80% of the consumed energy after critical illness caused by the trauma.
3. Appropriate diet supplements should be provided for the speedy recovery of the patient.

Multiple Choice Questions

Q1. Moderate acute malnutrition (MAM), defined as weight-for-height z-score (WHZ) between

A. –2 and –3
B. –4 and –5
C. Mid-upper arm circumference (MUAC) between 115 millimeters and <125 millimeters
D. A and C

Q2. Severe acute malnutrition (SAM), defined as

A. WHZ <–3
B. MUAC < 115 millimeters,
C. The presence of bilateral pitting edema
D. All of these

Q3. Which of the following are important sources of 3-omega fatty acids?

A. Walnuts, flaxseed
B. Papaya, oranges
C. Green leafy vegetables
D. All of these

Q4. RQ of carbohydrates is close to?

A. 2.0
B. 1.0
C. 0.5
D. 0.8

Q5. Which of these provide 9 kcal energy per gram?

A. Proteins
B. Carbohydrates
C. Lipids
D. Minerals

Q6. Dietary fibers consist mainly of

A. Cellulose
B. Dextrins
C. Mucopolysaccharides
D. Starch

Q7. The average daily requirement of protein is

A. 0.6–0.75 g/kg body weight
B. 6–7.5 g/kg body weight
C. 60–75 g/kg body weight
D. 0.6–0.75 mg/kg body weight

Q8. Specific dynamic action (SDA) for protein is

A. 5%
B. 13%
C. 30%
D. 60%

Q9. Which of these are not good sources of energy?

A. Proteins
B. Carbohydrates
C. Lipids
D. Fatty acids

Q10. Average kcals required from metabolic fuels for a 70 kg human adult is about

A. 100–200
B. 1000–2000
C. 2400–2900
D. 5000–7000

Q11. Which one of the following hormones is appetite suppressant?

A. Leptin
B. Glucagon
C. Insulin
D. Renin

Q12. Which of the following percentage of biological value indicates complete utilization of dietary protein?

A. 10
B. 50
C. 25
D. 100

Q13. Which of these are responsible for 1.0 net protein utilization?

A. Eggs
B. Milk
C. Cereals
D. A and B

Q14. Chemical score of egg protein is

A. 10
B. 50
C. 25
D. 100

Q15. Compromised intake of which vitamin is associated with neural tube defect in newborn baby?

A. Vitamin A
B. Folic acid
C. Ascorbic acid
D. Riboflavin

Q16. The total average iron loss of pregnancy is estimated at about

A. 100 mg
B. 500 mg
C. 1040 mg
D. 450 mg

Q17. The recommended iron intake of iron during pregnancy should be increased per day by

A. 10–25 μg
B. 9–29 mg
C. 9–27 μg
D. 100–250 mg

Q18. Goat milk lacks

A. Protein
B. Iron
C. Folic acid
D. B and C

Q19. All these are sources of complete proteins, *except*

A. Cereals
B. Meat
C. Milk
D. Eggs

Q20. All these are sources of incomplete proteins, *except*

A. Legumes
B. Fish
C. Cereals
D. Vegetables

Q21. Cereal proteins lack

A. Cysteine
B. Lysine
C. Threonine
D. B and C

Q22. Chemical score of meat is about

A. 10% B. 20%
C. 50% D. 70%

Q23. Biological value (BV) of rice is about

A. 70% B. 68%
C. 10% D. 20%

Q24. Net protein utilization of wheat is about

A. 90% B. 47%
C. 10% D. 25%

Q25. Protein efficiency ratio (PER) of rice is about

A. 4.5% B. 1.7%
C. 2.2% D. 1.5%

Q26. All these have high glycemic index *except*

A. Fructose B. Glucose
C. Maltose D. White rice

Q27. Tristearin has RQ

A. 1.0 B. 0.7
C. 1.5 D. 0.5

Q28. All these contain high concentration of cholesterol *except*

A. Meat B. Egg yolk
C. Cheese D. Pulses

Q29. Absorption of calcium is facilitated by

A. Vitamin D B. Lactose
C. High protein diet D. All of the above

Q30. All these factors interfere in the absorption of calcium in the intestine *except*

A. Phytic acid B. Vitamin D
C. Phosphates D. Oxalates

Q31. Which of these are richest sources of calcium?

A. Cereals B. Milk
C. Cheese D. B and C

Q32. In female, due to menstruation, the monthly loss of iron is about

A. 0.5–1.5 mg B. 1.5–8 μg
C. 150–280 mg D. 1.5–2.8 mg

Q33. The daily requirement of iron for hemoglobin synthesis in normal individuals is about

A. 2–5 mg B. 20–25 mg
C. 200–250 mg D. 20–50 μg

Q34. All of the following are chief dietary sources of iron *except*

A. Liver B. Green vegetables
C. Milk D. Egg yolk

Q35. All these are facilitatory substances for absorption of iron in the small intestine *except*

A. Tannin in tea B. Meat
C. Sugars D. Citric acid

Q36. Iron can be lost in the female athlete by all of the following *except*

A. Hair
B. Foot-strike destruction of red blood cells
C. Iron loss during menstruation
D. Sweat

Q37. Lack of which of these in the human body causes a severe deficiency disease called kwashiorkor?

A. Proteins B. Carbohydrates
C. Lipids D. Vitamins

Q38. Normal BMI is

A. 25–29 B. 12–16
C. 18.5–24.9 D. 29–34

Q39. A 28-year-old pregnant woman was found to be anemic in routine examination. Her physician is expected to

A. Start iron and vitamin therapy immediately
B. Start iron, vitamin and food therapy, only after diagnosing the type of anemia
C. Start only food therapy
D. A and C

Q40. A 30-year-old pregnant woman was found to be anemic with β-thalassemia trait in routine examination. Her physician is expected to

A. Start iron and vitamin therapy immediately
B. Start iron, vitamin and food therapy, only after diagnosing the type of anemia
C. Start only food therapy
D. Direct her husband for determination of any abnormal Hb
E. B and C

Q41. A six-month-old baby was found to be in distress following breast feeding. There was no history of fever but significant weight gain was not observed lately. Physician should recommend which of the following test (or tests) to support his diagnosis?

A. Lactose in feces
B. Urine glucose
C. Uristix test
D. Both A and B

Answers

1.D **2.** D **3.** A **4.** B **5.** C **6.** A **7.** A **8.** C **9.** A **10.** C
11. A **12.** D **13.** D **14.** D **15.** B **16.** C **17.** B **18.** D **19.** A **20.** B
21. D **22.** D **23.** B **24.** B **25.** C **26.** A **27.** B **28.** D **29.** D **30.** B
31. D **32.** A **33.** B **34.** C **35.** A **36.** A **37.** A **38.** C

39. B: Since, to treat anemia, it is necessary to first find out basic cause of anemia.

40. D: Since thalassemia trait could be asymptomatic; if anemia is not indicated by CBC test. Hence, it is necessary that her husband should test his blood to find out, whether he is also carrying thalassemia trait. When both the parents have thalassemia trait, there are 50% chances that a child may have thalassemia major.

41. A: Since the child appears to be in distress following breast feeding, it is due to lactose intolerance, due to deficiency of the enzyme lactase. When lactase is not digested, it gets fermented in the intestine and gases produced in fermentation cause discomfort to the child.

Case Studies

Case 1: A two-year-old boy presented with stunted growth, pitting edema, anorexia, ulcerating dermatoses, and an enlarged liver. His hemoglobin was 6.5 g/dl (Normal: 11.5–13.5), serum total proteins: 4.5 g/dl (Normal: 6–8 g/dl) and albumin: 2.0 g/dl (Normal: 3.3–4.5 g/dl).

Q1. What is the diagnosis?

Ans: Very low hemoglobin, serum total proteins and albumin with the presence of pitting edema indicate that the boy is suffering from **kwashiorkor.**

Q2. What is the biochemical basis of this case?

Ans: Poor nutrition is associated with a significant decreased concentration of plasma proteins. Deficiency of micronutrient and antioxidants are also contributory factors.

Q3. What is the treatment?

Ans: The patient is treated through three stages: Rehydration, stabilization and nutritional rehabilitation and follow-up:

1. **Rehydration:** The patient is treated with rehydrating solution containing electrolytes, glucose, amino acids, vitamins, and minerals by mouth or by vein.
2. **Stabilization:** Liquid diet is provided with approximately 80–100 kilocalories per kilogram per day (kcal/kg/d) spread over 8–12 meals per day for three to seven days.
3. **Nutritional rehabilitation and follow-up:** Locally available, nutrient-dense foods containing multi-vitamins and minerals to improve nutritional status and weight.

Case 2: A three-year-old girl presented with fever, severe headache, stunted growth, skin legions, loss of hair and an enlarged liver. Her physical examination observations were, WHZ < –3 and MUAC < 115 millimeters. Her hemoglobin was 5.6 g/dl (Normal: 11.5–13.5), serum total proteins: 4.3 g/dl (Normal: 6–8 g/dl) and albumin: 2.0 g/dl (Normal: 3.3–4.5 g/dl).

Q1. What is the diagnosis?

Ans: Severe acute malnutrition (SAM). It is defined as WHZ < 3 and MUAC <115 millimeters, with significant decreases in hemoglobin, serum total proteins and albumin.

Q2. What is the biochemical basis of this case?

Ans: Poor nutrition associated with a significant decreased concentration of hemoglobin and plasma proteins. Deficiency of micronutrient and antioxidants are also contributory factors.

Q3. What is the treatment?

Ans: The patient is treated through three stages: Rehydration, stabilization and nutritional rehabilitation and follow-up as explained in Case 2.

BAQ: Show horizontal integration of symptoms and test reports of Case 1 and 2 patients with anatomy, physiology and biochemistry.

Ans: Horizontal integration of severe malnutrition with pre-medical subjects.

Anatomy: Poor development of the body due to severe malnutrition. Affected blood cells, and various organs such as liver, kidneys and organs of gastrointestinal tract. Affected immune system due to low available amino acids-proteins.

Physiology: Affected functions of red blood cells and serum proteins due to significantly decreased concentrations: Transfer of water from blood to tissue spaces due to very low serum albumin and total proteins.

Biochemistry: Disturbed metabolism of amino acids and protein synthesis.

BAQ: Show vertical integration of symptoms and test reports of Case 1 and 2 patients with general medicine, clinical pathology, preventive medicine and pediatrics.

Ans: Vertical integration of severe malnutricion with para-clinical subjects

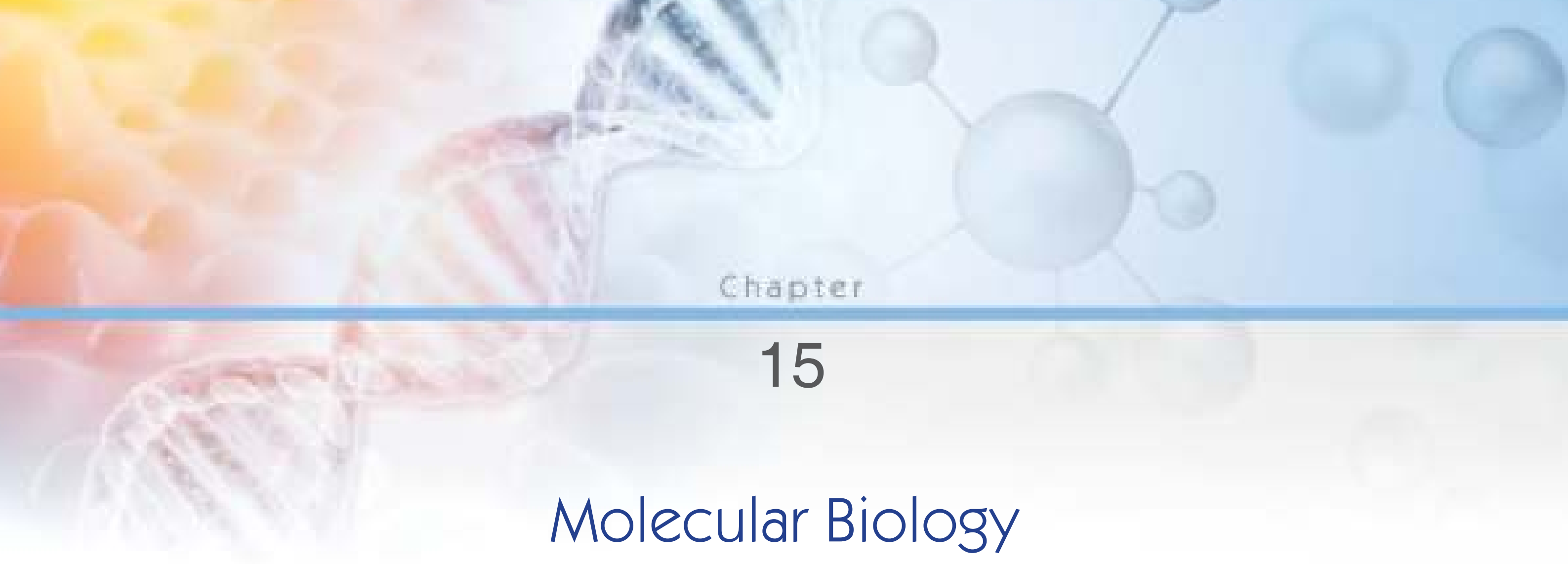
General medicine: Study of severe protein-energy malnutrition.

Clinical pathology: Determination of cause of the disease and related prognosis using laboratory tests to find out level of hemoglobin and plasma proteins.

Preventive medicine: Study of prevention of malnutrition in children.

Vertical integration of severe malnutrition with clinical subject

Pediatrics: Study of nutritional aspects in the case of children to prevent and treat severe protein-energy malnutrition.



Chapter

15

Molecular Biology and Pathology

SAQ: Short answer question: Marks: 2
BAQ: Brief answer question: Marks: 4 or 5
LAQ: Long answer question: Marks: 9 or 10
MCQs: Marks: 0.5 each
Case Studies: Marks: 4 or 5

INTRODUCTION

Molecular biology is the branch of biology that deals with the analysis of the structure and development of biological systems concerning the chemistry and physics of their molecular constituents. It is the study of molecular structures and events underlying biological processes, including relationships between genes and the functional characteristics they determine. Deoxyribonucleic acid (DNA) present in the nucleus of a specific cell carries genetic information from generation to generation. DNA is responsible to preserve the identity of a species for several years. The central dogma of life means the flow of biological information from DNA to ribonucleic acid (RNA) and from RNA to proteins.

Molecular pathology refers to the analysis of nucleic acids to diagnose disease, guide therapy, and evaluate susceptibility to the disease before the disease is evident. Molecular pathologic techniques provide new insights into clinical diagnosis, and they are complementary to traditional laboratory tests. By molecular pathology techniques, it is possible to determine the sequence of the human genome.

MOLECULAR COMPOSITION AND STRUCTURE OF DNA AND RNA

Competency achievement: The student should be able to:

BI7.1: Describe the structure and functions of DNA, and RNA, and outline the cell cycle

LAQ: Describe the cell cycle.

Ans: A cell cycle is a series of events that takes place in a cell during its growth and division through various stages such as interphase, prophase, prometaphase, metaphase, anaphase, and telophase (Fig. 15.1). Cytokinesis is the final physical cell division that follows telophase. The basic function of the cell cycle is to accurately duplicate DNA in the chromosomes followed by segregation of the copies of DNA precisely into two genetically identical daughter cells.

In the interphase, the cell gets itself ready for mitosis. The majority of eukaryotic cells spend most of their time in interphase. Interphase is a phase of active preparation for cell division, and it is not the first stage of mitosis. However, since mitosis is the division of the nucleus, prophase is the first stage. In interphase, the cell obtains nutrients, grows, reads its DNA, makes a copy of DNA, and conducts other normal cell functions.

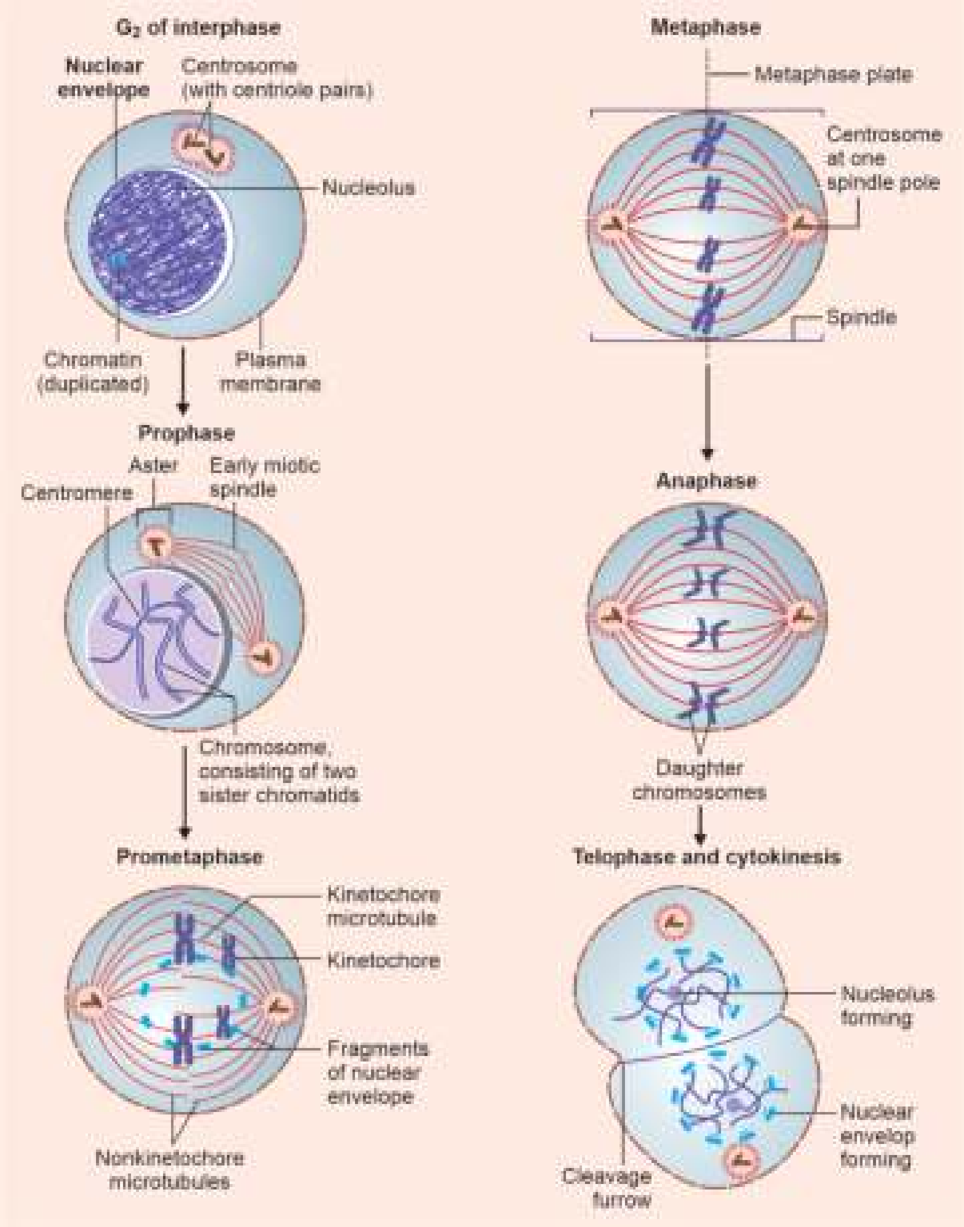

G₂ of interphase
Nuclear envelope
Centrosome (with centriole pairs)
Nucleolus
Chromatin (duplicated)
Plasma membrane
Prophase
Aster
Early miotic spindle
Centromere
Chromosome, consisting of two sister chromatids
Prometaphase
Kinetochore microtubule
Kinetochore
Fragments of nuclear envelope
Nonkinetochore microtubules
Metaphase
Metaphase plate
Centrosome at one spindle pole
Spindle
Anaphase
Daughter chromosomes
Telophase and cytokinesis
Nucleolus forming
Nuclear envelop forming
Cleavage furrow

Fig. 15.1: Various stages of mitosis

There are three stages of interphase. Each phase ends when a cellular checkpoint confirms the accuracy of the completion of a specific stage before proceeding to the next stage. The stages of interphase are as follows:

1. **G1 (Gap 1):** In this stage, the cell grows and functions normally. During this time, protein synthesis occurs, and the cell grows.
2. **Synthesis (S):** In this stage, the cell duplicates its DNA (via semiconservative replication).

3. **G2 (Gap 2):** In this stage, the cell resumes its growth in preparation for mitosis.

Various stages of mitosis are as follows (Fig. 15.1):

- **Early prophase:** In the early phase of prophase, the centrosome is duplicated, and the separated parts of chromosomes take their positions at the opposite pole.
- **Prophase:** In this stage, the two centrosomes, which migrate to the opposite poles of the cell, remain attached by fine spindle-like threads. The chromatin becomes concentrated and forms rod-shaped chromosomes. Nucleoli disappear at this stage.
- **Prometaphase:** In this phase, chromosomes attain maximum thickness and minimum length. Each chromosome forms two kinetochores (specific ring structure) at the centromere, one attached at each chromatid.
- **Metaphase:** In metaphase nuclear membrane disappears. Chromosomes arrange themselves around the center of the cell and appear to be attached to the spindle of the centrosome.
- **Anaphase:** In anaphase, the two groups of chromosomes begin to move away from each other. They arrange themselves around the centrosome, and the spindle breaks.
- **Telophase:** In this phase, a constriction appears around the center of the cell body. The nuclear membrane reappears, and the spindle disappears. The constriction of the cytoplasm increases until the cell is divided. The chromosomes disappear and, thread-like chromatin reappears and the two new cells formed are known as the daughter cells. These cells grow and reproduce by mitosis.

Cytokinesis

Cytokinesis is the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells. It is the division of the cytoplasm. It usually initiates during the late stages of mitosis, splitting a binucleate cell in two, to ensure that chromosome number is maintained from one generation to the next.

Cell Cycle and Maintenance of Integrity of DNA

In higher organisms, the cell cycle consists of four distinct phases such as mitotic, G1, S, and G2. Replication of DNA occurs only once in S-phase. The entire process of new DNA synthesis takes place in about 8–10 hours.

Normal survival of a species depends on the integrity and maintenance of DNA. Due to specific environment factors and replication errors, DNA may get damaged. If the damaged DNA is not repaired, it may lead to mutation or cell death.

LAQ: Describe the structure of DNA and RNA.

Ans: Structure of deoxynucleic acid (DNA) (Watson and Crick model)

- DNA is a polymer made up of deoxynucleotides. It is composed of monomeric units such as dAMP, dGMP, dCMP, and dTMP.
- The monomeric deoxynucleotides in DNA are held together by 3′, 5′-phosphodiester bridges (Fig. 15.2).

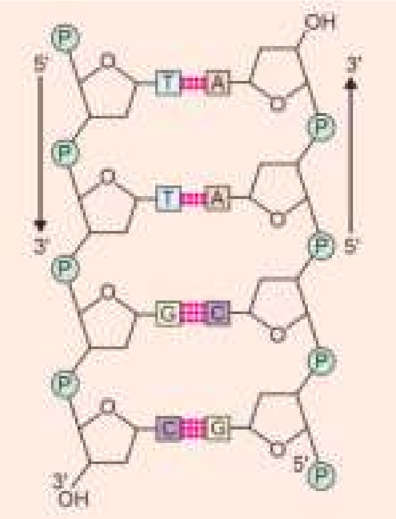


Fig. 15.2: Base pairing of DNA

- The DNA is a right-handed double helix (as proposed by Watson and Crick). It consists of two antiparallel right-handed double helix in which one strand runs in 5′5′ to 3′3′ direction while the other in 3′3′ to 5′5′ direction.
- The two strands are held together by hydrogen bonds formed by complementary base pairs. The A–T pair has 2 hydrogen bonds, while G–C pair has 3 hydrogen bonds.
- In all the species, the DNA has equal numbers of adenine and thymine residues and equal numbers of guanine and cytosine residues.
- DNA double helix described by **Watson and Crick** is the most predominant form under physiological conditions and is known as **B-form.**
- In a human cell, there are 46 chromosomes (23 pairs) which contain about 100,000 genes.
- A total length of DNA per cell of 1–2 meters is packed into a nucleus, millions of times smaller in diameter.
- An adult human body has about 10^{14} cells.

Thus, the total length of DNA in the human body is about 2×10^{10} km, i.e., 20 billion km.

Structure of Ribonucleic Acid (RNA) (Figs15.4 and 15.5)

- RNA is a polymer of ribonucleotides held together by 3′, 5′-phosphodiester bridges. The sugar in RNA is ribose. RNA contains the pyrimidine uracil in place of thymine (in DNA).
- RNA is usually a single-stranded polynucleotide. However, this strand may fold at certain places to give a double-stranded structure, if complementary base pairs are in close proximity.

The following different types of RNAs are present in the nucleus and cytoplasm:

1. **mRNA:** Messenger RNA is a single-stranded molecule. It is a copy of only one of the two strands of DNA of a gene. It specifies the sequence of amino acids in the protein synthesis (translation) (Fig.15.4).
2. **rRNA:** Ribosomal RNA are the main site of protein synthesis. They are composed of 60S and 40S subunits. The 60S subunit contains 28S, 5S, and 5.85S subunits, while

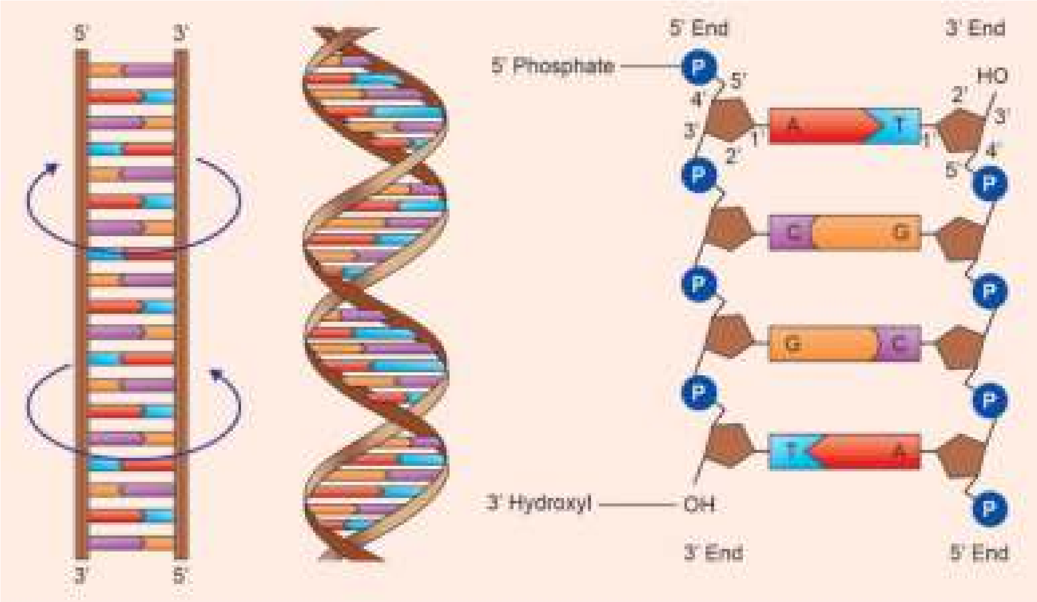


Fig. 15.3: Molecular structure of DNA

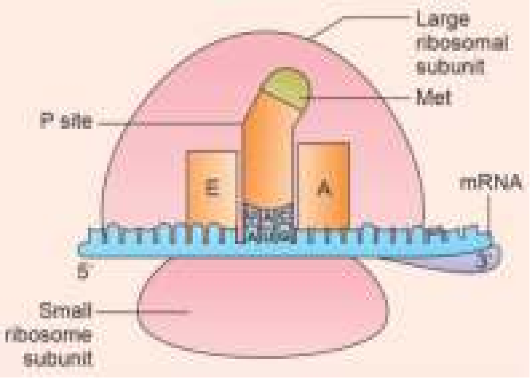


Fig. 15.4: rRNA

40S subunit contains 18S subunit. rRNA plays a significant role in the binding of mRNA to ribosomes and protein synthesis. 60S and 40S subunits combine to form the 80S functional ribosome. The prokaryotic functional ribosome is 70S (a combination of subunits 50S and 30S).

3. **tRNA:** The presence of several intra-chain hydrogen bonds give tRNA a clover-leaf-like shape (Fig. 15.5). tRNA molecule is folded in the following four loops:
    1. *The D loop and arm:* It contains a dihydrouridine (DHU) base. D loop is used for proper recognition of a specific tRNA by its aminoacyl-tRNA synthetase enzyme.
    2. *The T Ψ C loop and arm:* This loop contains an uracil nucleotide-pseudouridine with a carbon-carbon bond between the sugar and the base. The arm is used in the binding of aminoacyl-tRNA to the ribosome.
    3. Anticodon arm: It carries the specific "anticodon" triplet for recognizing binding-site on the ribosome.
    4. The variable loop: This is present between T Ψ C arm and the anticodon arm and varies according to a specific tRNA.
    5. The 3′-ACC-amino acid acceptor end: It carries a specific amino acid to be transferred into the ribosomes for protein synthesis. About 33 types of tRNA are found in the cytoplasm and about 22 types in mitochondria.

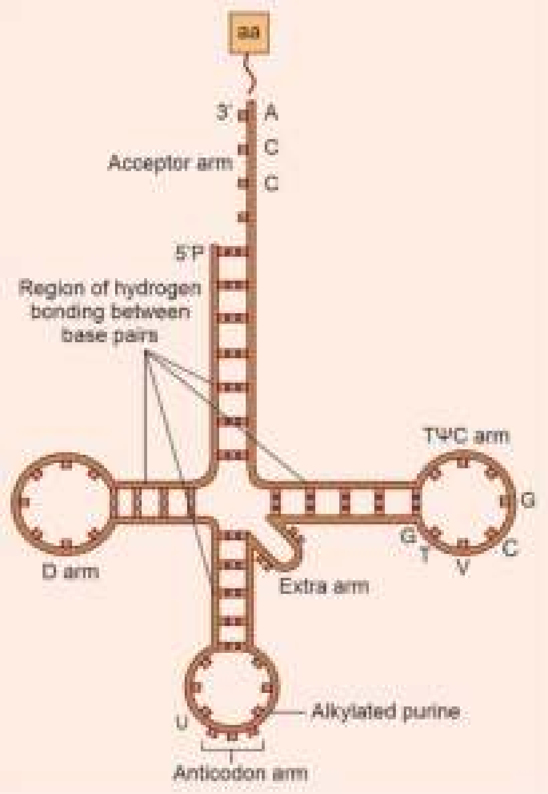


Fig. 15.5: tRNA

BAQ: Enumerate functions of nucleic acids.

Ans: The following are the various functions of nucleic acids:

1. DNA is present in the nucleus of eukaryotic organisms, and it is also found in the mitochondria. Nucleic acids play a very important role in the storage and expression of genomic information. DNA is the chemical basis of heredity and may be regarded as the reserve source of genetic information. DNA is exclusively responsible for maintaining the identity of different species of organisms.
2. Every aspect of cellular function is under the control of DNA, which it performs by using specific stretches on it, known as genes. The genes control the protein synthesis through the mediation of RNA.

3. **The central dogma of molecular biology** states that DNA contains instructions for protein synthesis, which are copied by RNA, and then these instructions are used to make a specific protein, as shown below:

$$\text{DNA}\xrightarrow{\left(\text{Transcription}\right)}\text{mRNA}\xrightarrow{\left(\text{Translation}\right)}\text{Protein}$$

SAQ: State Chargaff's rule.

Ans: Chargaff's rules state that

1. In the DNA of any organism and any species, the amount of adenine should be equal to the amount of thymine and the amount of guanine should be equal to the amount of cytosine.
2. A 1:1 stoichiometric ratio of purine and pyrimidine bases (i.e. A+G = T+C) should exist.

REPLICATION, TRANSCRIPTION AND TRANSLATION MECHANISMS

Competency achievement: The student should be able to:

BI7.2: Describe the processes involved in replication and repair of DNA and transcription and translation mechanisms

REPLICATION

LAQ: Write a note on replication of DNA.

Ans: Replication of DNA (Fig. 15.6):

Each time a cell divides, the entire DNA content of that cell must duplicate to retain the total complement of hereditary information (the genome) in each daughter cell. This process is called replication. Owing to the law of base pairing (i.e., adenine pairs only with thymine, and guanine only with cytosine), the sequence of a single strand of DNA dictates the sequence of its complementary strand. The DNA duplication process is known as semiconservative replication. The replication products consist of two dsDNA molecules, composed of one parent strand and one daughter strand each, with exactly the same base-pair sequence. This process is complicated and involves a number of accessory proteins and enzymes. The replication of the DNA of prokaryotes takes place as follows:

1. **Separation of two complementary DNA strands** (Fig. 15.6)**:** Replication of DNA is initiated at multiple sites. Each origin of replication is used only once during a single cell cycle. The formation of a single-stranded region is not energetically favorable, and it is effected by proteins that unwind and separate the strands of the helix. The two complementary strands of DNA separate at the site of replication to form a bubble. Multiple replication bubbles are formed in the eukaryotic DNA molecule.
2. **Formation of the replication fork** (Fig. 15.6)**:** The two strands unwind and form a "V" where active synthesis occurs. This region is called the replication fork. Replication of double-stranded DNA is bidirectional.
3. **Chain elongation**: Daughter strands are synthesized by DNA polymerase III, an enzyme that reads the parent template and attaches nucleotides to the growing daughter strand according to the base-pairing rules of dsDNA. DNA polymerase III acts at the point of strand separation, with a short RNA primer that base-pairs to the parent template. Later, this primer is excised and replaced with DNA by the DNA repair enzyme, DNA polymerase I.

Chromosomal DNA contains many initiation sites for replication and this process occurs simultaneously across the chromosome. DNA polymerase III is a directional enzyme and can synthesize DNA only in the 5′5′ to 3′3′ direction because it requires a free 3′OH end. Hence only one daughter strand, called the leading strand, can be synthesized continuously. The opposite strand is called the lagging strand. It is primed by RNA primase that does not require a free

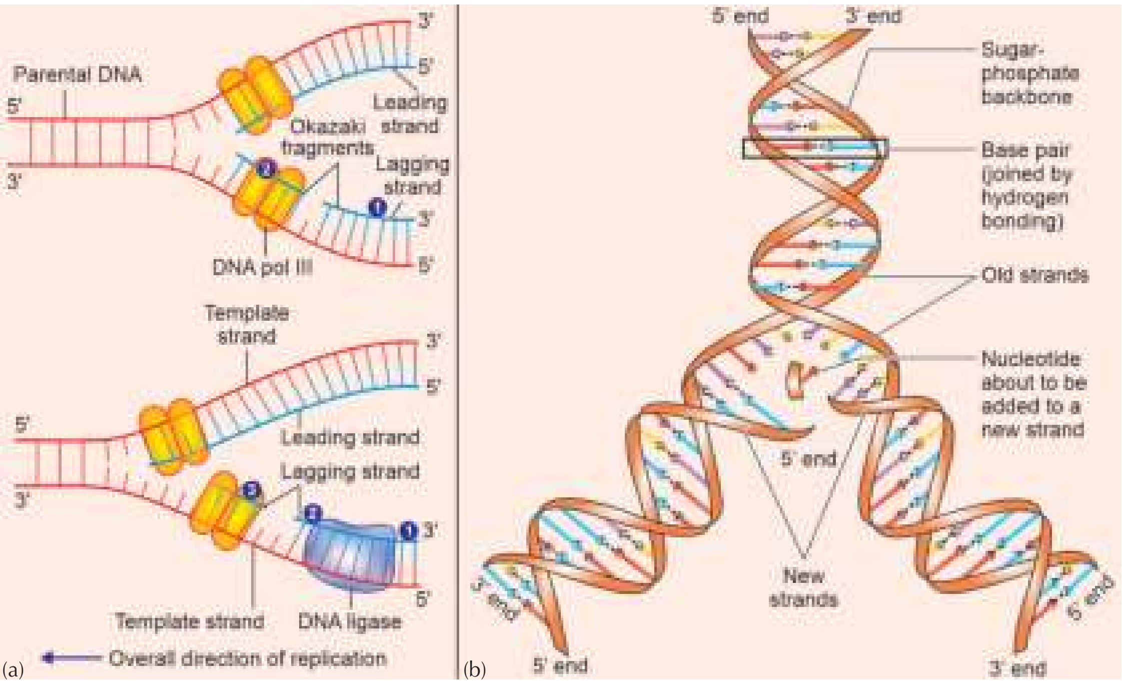

Parental DNA
5'
3'
3'
5'
Leading strand
Okazaki fragments
Lagging strand
3'
5'
DNA pol III
Template strand
3'
5'
5'
3'
Leading strand
Lagging strand
3'
5'
Template strand
DNA ligase
Overall direction of replication
(a)
(b)
5' end
3' end
Sugar-phosphate backbone
Base pair (joined by hydrogen bonding)
Old strands
Nucleotide about to be added to a new strand
5' end
New strands
3' end
5' end
5' end
3' end
5' end

Fig. 15.6: (a) Origin of replication (b) DNA replication

3’OH end. It is synthesized discontinuously in short fragments (Okazaki fragments) as the replication fork is opened up. These Okazaki fragments are then joined together by DNA ligase. DNA polymerase III also performs proofreading and exonuclease activity. The introduction of incorrect nucleotide is detected and excised by the nuclear portion of the enzyme, and the correct nucleotide is added.

The process of eukaryotic DNA replication closely follows that of prokaryotic synthesis. In the case of prokaryotes, the single origins of the replication of DNA are seen; however, in the case of eukaryotes, multiple origins of DNA replication are seen. In the case of eukaryotes, the various DNA polymerases that take part in DNA replication are, Pol α (synthesis of RNA primer), Pol β (repair of DNA), Pol ε (lagging strand synthesis and proofreading), Pol δ (replication of the leading strand) and Pol γ (participation in replication of mitochondrial DNA).

The following various proteins and enzymes play a very important role in the DNA replication:

- **DNA protein:** This ATP-requiring protein causes the double-stranded DNA to melt, i.e. the strands separate forming localized regions of single-stranded DNA.
- **Single-stranded DNA-binding (SSB) proteins:** These proteins keep the two strands of DNA separated in the area of the replication origin. These proteins also protect the DNA from nucleases that cleave single-stranded DNA.
- **DNA helicases:** These enzymes bind to single-stranded DNA near the replication fork and then move into the neighboring double-stranded region and perform the unwinding of the double helix.

SAQ: What are genes and their functions?

Ans: The information in DNA is arranged in units specifying the production of proteins and RNA molecules required for cellular function. These units are called genes. Genes include coding regions specifying the amino acid sequence of a protein and the regulatory regions controlling the rate and timing of the production of a specific protein. One gene contains the amino acid sequence code for one protein as well as DNA sequences necessary for the regulation of the production of that protein.

Although gene coding sequences are of paramount importance to the cell and the function of the organism, the vast majority of the human genome is not composed of genes. The non-coding DNA regions are sometimes called junk DNA, and their functions are not very well understood.

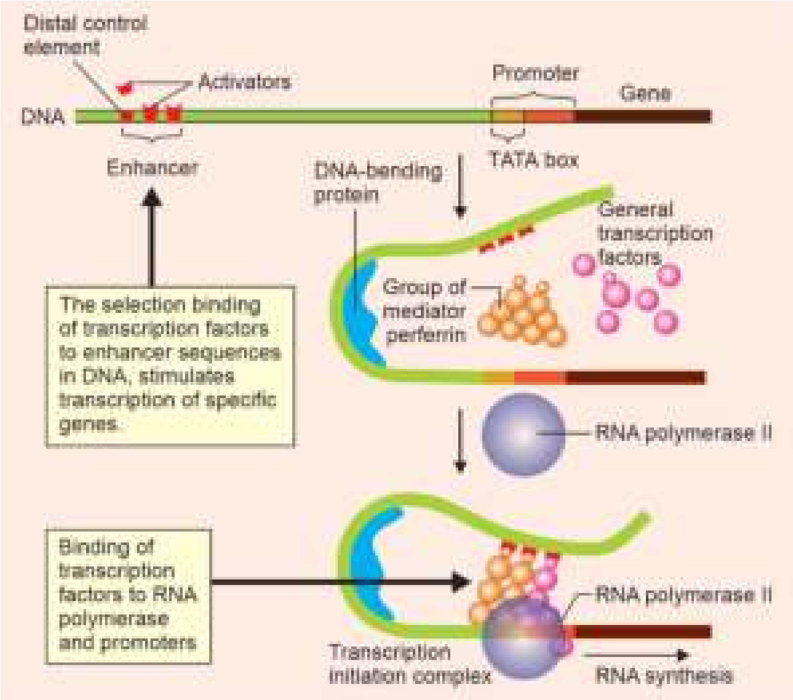
TRANSCRIPTION

LAQ: Write a note on the transcription of DNA to RNA (Figs 15.7–15.9).

Ans: Protein synthesis begins with the activation of the appropriate gene. A copy of the gene is made from DNA in the form of mRNA, which carries the code from the DNA in the cell nucleus to the cytoplasm, where amino acid synthesis takes place. mRNA is synthesized from only one strand of the DNA gene. The complementary DNA strand is not used. This is accomplished by a process called transcription.

Transcription: Like replication, transcription also requires the separation of the duplex DNA strands. In this process, DNA-dependent RNA polymerase binds to sequences in the regulatory region of the gene called the promoter. Promoters are usually rich in thymine (T) and adenine (A) in repeating patterns and have been referred to as a TATA box (Hogness box) (Fig. 15.7). Between 70–80 nucleotides upstream from the transcriptional start site is a second consensus sequence known as the CAAT box. One or both of these sequences may serve as recognition sites in eukaryotic promoters.

The promoter site in the case of a prokaryotic cell is known as “the “Pribnow box”, which


Distal control element
Activators
Promoter
Gene
DNA
Enhancer
TATA box
DNA-bending protein
General transcription factors
Group of mediator perferrin
RNA polymerase II
RNA polymerase II
Transcription initiation complex
RNA synthesis

The selection binding of transcription factors to enhancer sequences in DNA, stimulates transcription of specific genes.

Binding of transcription factors to RNA polymerase and promoters

Fig. 15.7: Activation of DNA transcription

is a stretch of 6 nucleotides (TATAAT). Promoters occur approximately 100 bases "upstream" (i.e. at the 5′ end) from the initiation site of transcription where the first nucleotide unit is paired with the template (uracil pairs with adenine). Initiation of transcription requires many cofactors. These cofactors bind to RNA polymerase to form the active initiation complex. Regulation of transcription is the primary mechanism cells use to control gene expression.

DNA polymerase requires a primer for its action, but RNA polymerase acts without a primer. RNA polymerase does not possess endonuclease or exonuclease activity, hence it cannot repair the errors in RNA synthesis. However, mistakes in RNA synthesis are not transmitted to the daughter cell.

Transcription continues until chain termination occurs in response to specific Rho (σ) factor. The RNA transcript quickly detaches from the template DNA. The end product is a complementary sequence of ribonucleotides that contains the information necessary for protein synthesis. Additional modifications of mRNA are formed as follows:

1. The 5′ end is modified by the addition of 7-methyl guanosine residues to form a structure called the cap.
2. The 3′3′ end is modified by the addition of multiple adenine bases, called the poly-A tail, which stabilizes the mRNA molecule. Both caps and tails are necessary for the translation of mRNA into a specific protein.
3. Non-coding regions termed introns are excised from the mRNA by a molecular complex termed a spliceosome, which is composed of multiple small nuclear ribonuclear protein particles (snRNPs). Spliceosomes mediate the cleavage and ligation of RNA at specific recognition sequences.
4. After the removal of introns, the mRNA contains exons, i.e. coding sequences. mRNA is then transported into the cytoplasm and attaches to a small unit of sRNA (Fig. 15.8).

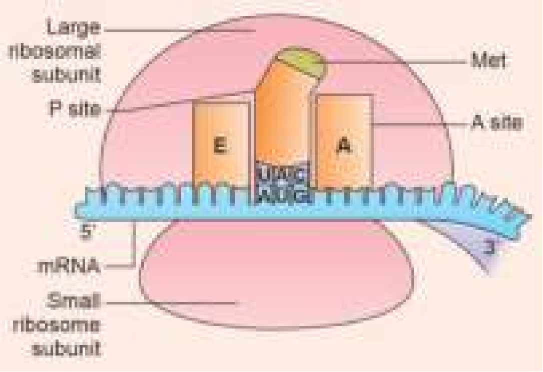


Fig. 15.8: Attachment of mRNA with the small subunit of the ribosome

TRANSLATION

The translation is a process, whereby the mRNA sequence directs the amino acid sequence during protein synthesis. In protein synthesis, twenty-one amino acids are involved and each is specified by a three-nucleotide sequence known as a codon. The codons are usually presented in the messenger RNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5′5′-end to the 3′3′-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time (4^3).

The assignment of codon triplets to amino acids is complete, and the list constitutes the genetic code. For example, the combination UUU means phenylalanine. Three codons have been reserved as 'stop' signals that indicate to the protein synthesizing machinery that the protein is complete. These codons are UAA, UAG, and UGA. These have no amino acids assigned to them in the genetic code and are known as nonsense codons. Of the remaining 61 codons, all code for amino acids. Only two amino acids, methionine, and tryptophan, have single codons, and the rest have more than one codon.

Translation takes place on the ribosome, which are ribonucleoprotein complexes that function as protein synthesis factories

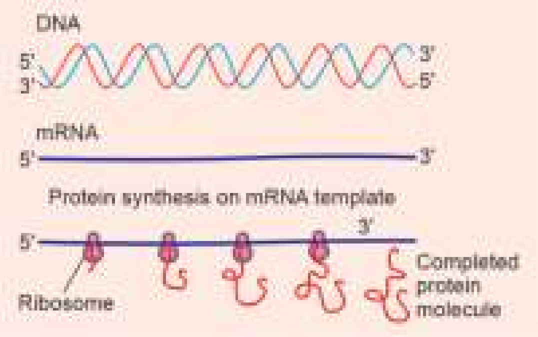

DNA
5'
3'
3'
5'
mRNA
5'
3'
Protein synthesis on mRNA template
5'
3'
Ribosome
Completed protein molecule

Fig. 15.9: Protein synthesis

(Fig. 15.9). A ribosome binds to the initiation site on mRNA to form an initiation complex. During protein synthesis, codons are "read" by transfer RNA (tRNA). tRNA molecules have a sequence complementary to an amino acid molecule specified by the codon. During elongation, the ribosome moves from the 5′′ to 3′ end of the mRNA. This process is known as "translocation". In prokaryotes, the elongation factors, EF-Tu and EF-Ts along with GTP, facilitate the elongation procedure. In the case of eukaryotes, translocation requires the factor eIF-2 and GTP.

In the beginning, the initiation occupies the "P site" of the ribosome. Next incoming tRNA-amino acid complex gets associated with the "A site" of the ribosome (Refer to Fig. 15.4).

As synthesis proceeds, the appropriate tRNA anticodon base-pairs with the next mRNA codon. An enzyme peptidyl transferase on the ribosome then catalyzes the formation of a peptide bond between the amino acid bound to the tRNA and the growing protein chain (Fig. 15.9). The previous tRNa is released, and the next tRNA is added. The ribosome moves along the mRNA until a stop codon (UAA, UAG, or UGA) is reached, and synthesis is complete. The release factors, RF-1, RF-2, and RF-3, bind with the stop codon and cause hydrolytic breakage of the polypeptide, and the ribosome and the protein products are then dissociated from the mRNA.

Folding of Polypeptide into Quaternary Formation

BAQ: Write a note on the folding of the polypeptide into quaternary formation.

Ans: After the release of the polypeptide, various "chaperones" (specific proteins) stabilize the polypeptide by binding at specific sites on the polypeptide, which require ATP molecules. The polypeptide gets folded by the interactions of various amino acids and by the actions of chaperones. The Golgi complex receives the stabilized and folded protein from the endoplasmic reticulum. The Golgi apparatus modifies the protein by the addition of carbohydrate, lipid, or sulfate moieties and, after sorting the protein, sends it out in a vesicle that delivers it to the appropriate place. Thus, the proteins and enzymes required for cellular function are retained within the cell, and other various types of proteins are sent across the plasma membrane to their respective destinations.

BAQ: What is genetic code? Write the importance of genetic code in the translation procedure in protein synthesis.

Ans: The genetic code is the sequence of nucleotides in DNA and RNA that determines the sequence of amino acids in the synthesis of proteins. During translation, the mRNA sequence directs the amino acid sequence during protein synthesis. In protein synthesis, twenty-one amino acids are involved and each is specified by a three-nucleotide sequence known as a codon. The codons are usually presented in the messenger RNA language of adenine (A), guanine (G), cytosine (C), and uracil (U). Their nucleotide sequences are always written from the 5′-end to the 3′-end. The four nucleotide bases are used to produce the three-base codons. There are, therefore, 64 different combinations of bases, taken three at a time (43).

The assignment of codon triplets to amino acids is complete, and the list constitutes the genetic code. For example, the combination UUU means phenylalanine. Three codons have been reserved as 'stop' signals that indicate to the protein synthesizing machinery that the protein is complete. These codons are UAA, UAG, and UGA. These have no amino acids assigned to them in the genetic code and are known as nonsense codons. Of the remaining 61 codons, all code for amino acids. Only two amino acids, methionine, and tryptophan, have single codons, and the rest have more than one codon.

SAQ: What is the meaning of the degeneracy in the genetic code?

Ans: "Degeneracy" in the genetic code means multiple codons decode the same amino acid. Some amino acids are encoded by several codons, e.g. six different codons specify serine. With rare exceptions, the genetic code is unambiguous, i.e. given a specific codon, only a single amino acid is indicated.

SAQ: What is the meaning of the fact that the genetic code is non-overlapping?

Ans: The reading of the genetic code during the process of protein synthesis does not involve any overlap of codons. Thus, the genetic code is non-overlapping. Once the reading is commenced at a specific codon, there is no punctuation between codons, and the message is read in a continuing sequence of nucleotide triplets until a nonsense codon is reached.

Q: What is the meaning of the fact that genetic code is universal?

Ans: With a few exceptions, the genetic code is universal. The genetic code can be universal which means, the same codons are used to code for the same amino acids in all living organisms.

SAQ: Name the following: Initiator codon and chain terminating codons in protein synthesis.

Ans: Initiator codon is AUG, which codes for methionine. Chain-terminating codons are UAA, UAG, and UGA. These have no amino acids assigned to them and are also known as nonsense codons.

SAQ: Name protein chain termination codons in mitochondria.

Ans: AGA and AGG serve as protein terminators in mitochondria.

SAQ: Write actions of (1) Actinomycin D and (2) Rifampicin on protein synthesis.

Ans:

1. The antibiotic Actinomycin D binds with the DNA template strand and blocks the movement of RNA polymerase.
2. Rifampin inhibits the activity of RNA polymerase by binding with the b-b-subunit of prokaryotic RNA polymerase.

SAQ: Write actions of (1) Teracyclin and (2) Chloramphenicol on protein synthesis.

Ans:

1. The antibiotic tetracycline binds to the 30S ribosomal subunit of prokaryotes and inhibits the binding of aminoacyl-tRNA to the A site of the ribosome.
2. The antibiotic chloramphenicol binds to the 50S ribosomal subunit and inhibits the peptidyltransferase enzyme that catalyzes peptide bond formation in protein synthesis.

SAQ: Write actions of (1) Erythromycin and (2) Mushroom toxin α-amanitin and (3) Neomycin on protein synthesis.

Ans:

1. Erythromycin binds to the 50S ribosomal subunit and prevents translocation.
2. Mushroom toxin α-amanitin inhibits transcription by binding with RNA
3. The antibiotic neomycin reduces the expression of all protein genes, inevitably leading to cell death.

SAQ: Write four characteristics of the genetic code.

Ans:

1. The genetic code is universal, i.e. the same codons are used to code for the same amino acids in all the living organisms
2. Genetic code is specific, i.e. a particular codon always codes for the same amino acid

3. Genetic code is non-overlapping, i.e. it is read as a continuous base sequence, without comma and any punctuation and degenerate.
4. There is "degeneracy" in the genetic code, i.e. multiple codons decode the same amino acid.

BAQ: Write a note on transcriptional control.

Ans: Transcription control takes place as follows:

1. The enhancers are DNA sequences that augment mRNA transcription and are found in different locations relative to the gene that they affect.
2. Transcription factors are proteins that bind to enhancers and promoters. These factors selectively stimulate or inhibit mRNA transcription.
3. Transcription factors, in turn, are controlled by hormones, growth factors, or cellular events such as phosphorylation.
4. A network of intra- and extra-cellular chemical communications can select and control the synthesis of necessary proteins.
5. mRNA is less stable than DNA, and its half-life is very short. New mRNA molecules are continually transcribed from DNA. The genes that are transcribed into mRNA can quickly change concerning the response of the cell to changes in transcriptional signals. This results in the immediate synthesis of new proteins.

LAQ: Write a note on the regulation of gene expression with examples.

Ans: Regulation of gene expression refers to the control of the amount and timing of the appearance of the functional product of a gene. Control of expression is vital to allow a cell to produce the gene products only when they are needed. This gives cells the flexibility to adapt to a variable environment, external signals, damage to the cell, etc.

Following are some examples of specifically required gene expression: Control of insulin expression so it gives a signal for blood glucose regulation and control of ADH for retention of water in the body or control of electrolytes by secretion of mineralocorticoids. In general, gene regulation gives the cell control over all structure and function and is the basis for cellular differentiation, morphogenesis, and the versatility and adaptability of any organism.

Any step of gene expression may be modulated, from the DNA–RNA transcription step to the post-translational modification of a protein. The stability of the final gene product, whether it is RNA or protein, also contributes to the expression level of the gene. An unstable product results in a low expression level. Following are various terms used to describe types of genes depending on how these are regulated:

1. A constitutive gene is a gene that is transcribed continually.
2. A housekeeping gene is typically a constitutive gene that is transcribed at a relatively constant level. The housekeeping gene's products are specifically needed for the maintenance of the cell. It is assumed that their expression is unaffected by experimental conditions.
3. A facultative gene is a gene that is only transcribed when needed.
4. An inducible gene is a gene whose expression is either responsive to environmental change or dependent on the position in the cell cycle.

Regulation of transcription follows three main routes of influence:

A. Genetic, i.e. direct interaction of a controlling factor with the gene,
B. Modulation, i.e. the interaction of a controlling factor with the transcription machinery, and
C. Epigenetic, i.e. non-sequence changes in DNA structure which influence transcription.

Direct interaction with DNA is the simplest and the most direct method by which a protein can change transcription levels. Genes often have several protein binding sites around the coding region with the specific function of regulating transcription. There are many classes of regulatory DNA binding sites known as enhancers, insulators, repressors, and silencers.

There are various mechanisms for regulating transcription. These range from blocking important binding sites on the DNA for RNA polymerase to acting as an activator and promoting transcription by assisting RNA polymerase binding.

The activity of transcription factors is further modulated by intracellular signals causing protein post-translational modification, including phosphorylated, glucosylated, or acetylated.

The nuclear membrane in eukaryotes allows further regulation of transcription factors by the duration of their presence in the nucleus which is regulated by reversible changes in their structure and by binding of other proteins. Environmental stimuli or endocrine signals may cause modification of regulatory proteins by producing cascades of intracellular signals, which result in the regulation of gene expression.

Expression of a gene coding for a protein is only possible if the messenger RNA carrying the code survives long enough to be translated. In eukaryotes, RNA is stabilized by certain post-transcriptional modifications, particularly the 5′5′ cap and poly-adenylated tail.

Once protein synthesis is complete, the level of expression of that protein can be reduced by protein degradation. There are major protein degradation pathways in all prokaryotes and eukaryotes. A protein that is not required or a damaged protein is often labeled for degradation by the addition of ubiquitin.

OPERON CONCEPT

SAQ: Write a brief introduction to the operon concept.

Ans: An operon is a functioning unit of genomic material. It contains a cluster of genes under the control of a single regulatory signal or promoter. The genes are transcribed together into an mRNA strand. These are either translated together in the cytoplasm or undergo trans-splicing to create monocistronic mRNAs that are translated separately. That means several strands of mRNA are formed that each encodes a single gene product. One example of an operon is the "lac operon", which was first studied in the *E. coli* organism. The operon (lac operon) is the coordinated unit of genetic expression in bacteria.

Control of an operon is a type of gene regulation that enables organisms to regulate the expression of various genes depending on environmental conditions. Operon regulation can be either negative or positive by induction or repression. Negative control involves the binding of a repressor to the operator to prevent transcription.

SAQ: What is the role of a promoter in transcription?

Ans: The promoter is recognized by RNA polymerase, which then initiates transcription. In RNA synthesis, promoters indicate which genes should be used for messenger RNA creation and thus controls the required protein synthesis.

SAQ: What is a negative inducible operon? Give an example.

Ans: In negative inducible operons, a regulatory repressor protein normally binds to the operator, and it prevents the transcription of the genes on the operon. If an inducer molecule is present, it binds to the repressor and changes its conformation so that it is unable to bind to the operator. This allows the expression of the operon. Example: Lac operon is negative inducible.

SAQ: What is a negative repressible operon? Give an example.

Ans: In negative repressible operons, transcription of the operon normally takes place. Repressor proteins are produced by a regulator gene, but they are unable to bind to the operator in their normal conformation. However, certain molecules called co-repressors bind by the repressor protein and cause a conformational change to the active state. The activated repressor protein binds to the operator and prevents transcription. Example: Tryptophan operon.

SAQ: What is a positively controlled operon?

Ans: In the case of a positively controlled operon, an activator protein stimulates transcription by binding to DNA, usually at a site other than the operator.

SAQ: What is a positively inducible operon?

Ans: In the case of positive inducible operons, activator proteins are normally unable to bind to the specific DNA. When an inducer is bound by the activator protein, it changes conformation so that it can bind to the DNA and activate transcription.

SAQ: What is a positive repressible operon?

Ans: In positive repressible operons, the activator proteins are normally bound to the specific DNA segment. However, when a co-repressor is bound by the activator, it is prevented from binding to the DNA. This stops the activation and transcription of the system.

LAC OPERON

BAQ: Write a note on lac operon.

Ans: The lac operon consists of the following genes: A regulatory gene (I), a promoter gene (P), where the enzyme RNA polymerase binds, an operator gene (O), and three structural genes (Z, Y, and A).

The promoter gene initiates the required metabolic process. Regulatory-terminator gene controls the energetically favorable metabolic pathway and terminates it when appropriate. The operational gene controls the overall operon system. Lac operon works in the following ways:

1. When lactose is available to *E. coli* bacteria, it binds with the lac repressor molecule (Fig. 15.10).
2. Lac repressor molecule becomes inactive and it does not bind the operator gene (O).
3. Now RNA polymerase can bind the promoter and initiates the transcription process and the operator gene (O) activates genes Z, Y, and A. Lac-Z encodes β-galactosidase, which cleaves lactose into glucose and galactose. Lac-Y encodes lactose permease, which facilitates the transport of lactose across the cell membrane of *E. coli* organisms. Lac-A encodes galactoside O-acetyl transferase, which transfers an acetyl group from acetyl-CoA to β-galactoside. *E. coli* use lactose molecules to derive ATP molecules for their activities. Lac-Z, lac-Y, and lac-A operate only when lactose is available to the organism; to derive energy in the form of ATP molecules (Fig. 15.10).
4. In the absence of lactose, when glucose molecules are available as a source of energy (ATP molecules), catabolite activator protein (CAP) acts as a glucose sensor.
5. The regulatory gene (I) is now used to express the synthesis of lac-repressor.
6. When regulatory gene (I) is expressed, "lac-repressor" is synthesized, which specifically binds to the operator gene (O). This prevents the binding of the enzyme RNA polymerase to the promoter site (P) and blocks the transmission of structural genes, Z, Y, A, and their respective functions.

The lac repressor is an allosteric protein (which can assume two shapes) present in two forms in equilibrium with each other. In one form, which is active, it binds to the operator gene (O). In another form, when it binds with lactose, it becomes inactive.

The lac repressor is an allosteric protein (which can assume two shapes) present in two forms in equilibrium with each other. In one form, which is active, it binds to the operator gene (O). In another form, when it binds with lactose, it becomes inactive (Fig. 15.10).

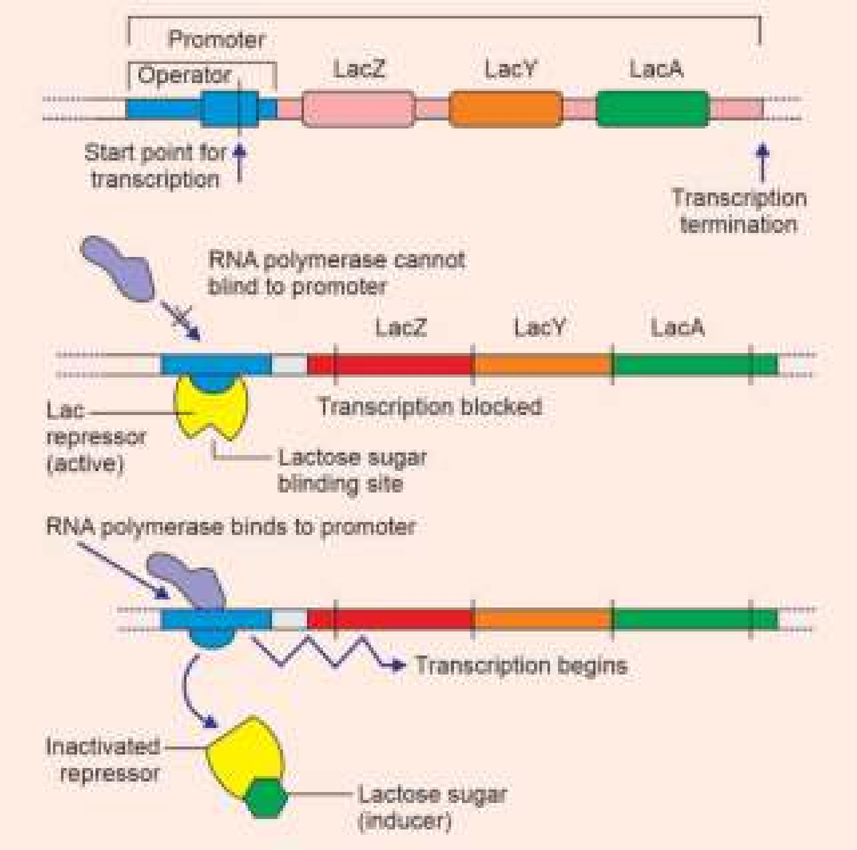

Promoter
Operator
LacZ
LacY
LacA
Start point for transcription
Transcription termination
RNA polymerase cannot blind to promoter
LacZ
LacY
LacA
Transcription blocked
Lac repressor (active)
Lactose sugar blinding site
RNA polymerase binds to promoter
Transcription begins
Inactivated repressor
Lactose sugar (inducer)

Fig. 15.10: The lac operon

TRYPTOPHAN OPERON

Q. Write a note on tryptophan operon.

Ans: Tryptophan operon contains the following components: Structural genes, primary internal promoter, operator, attenuator, secondary internal promoter, and terminator. The five structural genes are trp-E, trp-D, trp-B, trp-C, and trp-A. These genes code for three enzymes required for the synthesis of tryptophan from chorismate molecules. The primary promoter is trp-P, the operator gene is trp-O, the attenuator gene is trp-A, the secondary internal promoter is trp-P2 and the terminator is trp-T. The promoter trp-P of the tryptophan operon can bind to RNA polymerase. It also contains a repressor gene (trp-R) which synthesizes the repressor protein. Tryptophan operon functions as follows:

1. Tryptophan operon remains shut when tryptophan is available to the cells. Tryptophan acts as a co-repressor and binds to the repressor protein molecule. Tryptophan-bound repressor molecule then binds operator gene (O). This binding of tryptophan-bound repressor to operator gene prevents RNA polymerase from binding to the promoter and shuts down the operations of trp-E, trp-D, trp-B, trp-C, and trp-A and the synthesis of enzymes of tryptophan operon.
2. In the absence of tryptophan; when the repressor is inactive and not bound to the operator trp-O; RNA polymerase binds to the promoter and operator trp-O activates trp-E, trp-D, trp-B, trp-C, and trp-A genes for the synthesis of enzymes required to synthesize tryptophan from chorismate.

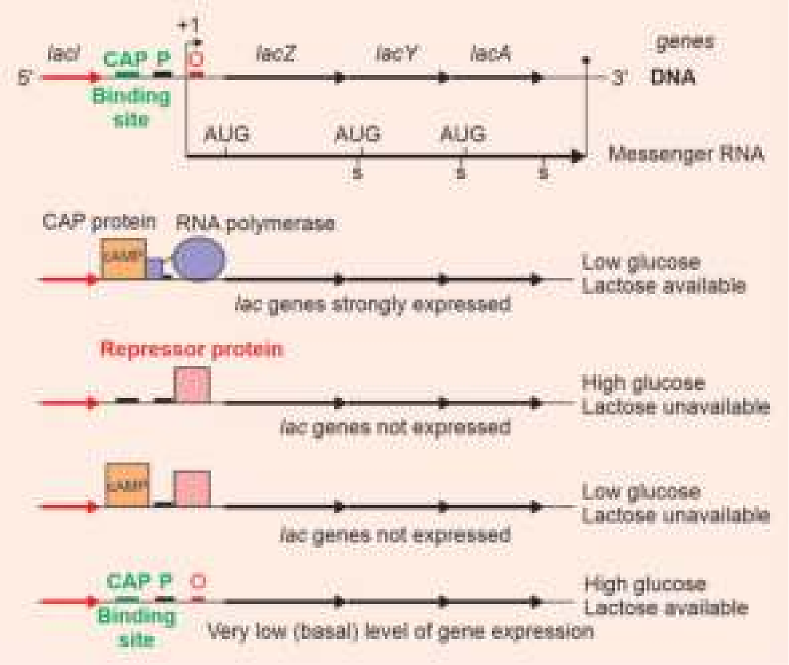

+1
lacI
CAP
P
O
lacZ
lacY
lacA
genes
5'
3'
DNA
Binding site
AUG
AUG
AUG
Messenger RNA
CAP protein
RNA polymerase
cAMP
Low glucose
Lactose available
lac genes strongly expressed
Repressor protein
High glucose
Lactose unavailable
lac genes not expressed
cAMP
Low glucose
Lactose unavailable
lac genes not expressed
CAP
P
O
Binding site
High glucose
Lactose available
Very low (basal) level of gene expression

Fig. 15.11: Tryptophan operon

3. Once sufficient tryptophan molecules are formed, tryptophan operon shuts down as described in step 1.

It is an example of negative regulation of gene expression. Within the operon's regulatory sequence, the operator is blocked by the repressor protein in the presence of tryptophan (thereby preventing transcription) and is liberated in tryptophan's absence (thereby allowing transcription). The process of attenuation complements this regulatory action.

MECHANISM OF DNA REPAIR

Damage to DNA during the normal cellular lifetime and errors in DNA replication must be minimized to preserve the health of the entire organism. Many mechanisms operate to maintain the normal DNA sequence. Error avoidance mechanisms operate during DNA replication. The DNA polymerase that synthesizes new DNA polymers selects each successive nucleotide monomer base on its complementarity to the next nucleotide in the template strand. Most errors in synthesis are avoided at this stage. Occasionally, if any base is added incorrectly to the growing strand, the proofreading activity of the polymerase can recognize the error. The incorrect base is then removed, and the new synthesis is continued. The error avoidance mechanisms reduce base-pair mismatches to approximately 1 in 10 million.

BAQ: Write a note on the DNA repair mechanism.

Ans: DNA can be damaged by normal biochemical reactions, and by non-physiological agents such as ultraviolet light and environmental carcinogens. Several repair mechanisms mend damaged DNA. The following are examples of DNA repair mechanisms:

Direct repair mechanisms repair lesions in a single-step reaction. For example, O-6-methylguanine DNA methyltransferase repairs alkylation lesions by transferring the alkyl group from the lesion to the active site of the enzyme.

Mismatch repair mechanisms function immediately after DNA replication to replace mismatched bases with the correct ones.

Mismatch repair proteins recognize the error in the newly synthesized daughter strand and excise a region that includes the mismatch. The correct sequence and integrity of the daughter strand are restored by DNA polymerase III and ligase.

Base excision repair mechanisms take care of small, non-helix deforming adducts such as those produced by oxidation, reduction, methylation, or base fragmentation by ionizing radiation. Base excision leaves 3–4 nucleotide sequence gaps that are then filled with the correct nucleotides. Nicks are sealed by the actions of ligases. The larger damages caused by UV radiation, carcinogens, and other agents, such as therapeutic drugs, are removed by the nucleotide excision repairs (NER) pathway. NER utilized an enzyme system composed of many proteins to excise a single-stranded oligonucleotide containing the lesion. The gap is then filled in by DNA polymerase and ligated. There are two nucleotide excision repair pathways. In the first pathway, the lesions that block transcription are rapidly removed, and in the second global pathway, the bulk DNA including the non-transcribed strand of active genes is repaired. The loss of NER activity is exemplified by the disease xeroderma pigmentosum (XP), which is caused by mutations in NER. XP results in extreme sensitivity to sunlight, with skin cancers occurring at an early age.

Double-strand break repair mechanisms take care of double-stranded breaks in DNA. Double-stranded breaks result from physiological processes or from ionizing radiation and oxidative insults. Double-stranded breaks are repaired by non-homologous or allelic-recombinational repair mechanisms.

GENE MUTATIONS

Competency achievement: The student should be able to:

BI7.3: Describe the gene mutations and basic mechanisms of regulation of gene expression

LAQ: Write a note on DNA mutations.

Ans: DNA mutations mean alterations in DNA base sequence. Mutations occurring in intron regions of DNA may not produce any adverse effect. DNA mutations occur despite repair mechanisms. To some extent, mutations must occur for the process of evolution to continue. For an individual organism, some mutations are harmful and can be associated with cancer and with inherited genetic diseases.

The following are various terms used to describe mutations:

- Lethal mutations are mutations that lead to the death of the organisms which carry the mutations. A harmful mutation is a mutation that decreases the fitness of an organism. A beneficial mutation is a mutation that increases the fitness of an organism or promotes desirable traits. A neutral mutation has no harmful or beneficial effect on the organism.
- Point mutations refer to an exchange of a single nucleotide for another nucleotide. These changes are classified as transitions or transversions. A point mutation is found in the β-globin gene in sickle cell anemia. A thymine base replaces an adenine base. Due to this replacement of the specific base (thymine), instead of glutamic acid in the primary structure of globin, valine is introduced, causing a critical change in the structure of hemoglobin, leading to sickle cell anemia.

Point mutations that occur within the protein-coding region of a gene may be classified into the following three types:

1. **Silent mutations:** which code for the same (or a sufficiently similar) amino acid. Hence, normal protein synthesis is not affected.
2. **Missense mutations:** which code for a different amino acid. This type of mutation may be acceptable or unacceptable, depending on the formation of a normal or abnormal protein product.

3. **Nonsense mutations:** which code for a stop and can truncate the protein. The end products are abnormal proteins as seen in the case of thalassemia disorders.

In transition type of gene mutations, there is an exchange of a purine for another purine (e.g. A→G) or a pyrimidine for another pyrimidine, (e.g. C→T).

In the transversion type of gene mutations, there is an exchange of a purine for a pyrimidine or a pyrimidine for a purine. An example of a transversion is adenine (A) being converted into cytosine (C).

In insertion mutations, one or more extra nucleotides are added to the DNA. They are usually caused by transposable elements or errors during the replication of repeating elements. An example is Fragile X syndrome. In fragile X syndrome, expression of the disease is associated with amplification beyond a certain limit of the number of intragenic trinucleotide repeat sequences.

Deletion in the gene may take place. An example is the disease muscular dystrophy, which is caused by deletions in the gene for the muscle protein dystrophin.

Frameshift mutations occur when there is a deletion or insertion of one or two nucleotides in DNA that generate altered mRNAs. In a frameshift mutation, insertions in the coding region of a gene may alter the splicing of the mRNA or cause a shift in the reading frame (frameshift), both of which can significantly alter the gene product. In a frameshift mutation, an entirely different protein product may form, or no protein formation may take place if a stop codon is encountered. An example is the disease, cystic fibrosis. In most cases of chronic myelogenous leukemia, the Philadelphia chromosome is found.

Reciprocal translocations are usually an exchange of material between non-homologous chromosomes.

A Robertsonian translocation results when the long arms of two acrocentric chromosomes fuse at the centromere and the two short arms are lost leading to trisomy 13.

SAQ: Write four diseases related to DNA mutations.

Ans:

1. Sickle cell anemia
2. Chronic myelogeneous leukemia,
3. Cystic fibrosis,
4. Muscular dystrophy.

MOLECULAR PATHOLOGY

BAQ: What is molecular pathology and the importance of molecular pathology techniques?

Ans: Molecular pathology refers to the analysis of nucleic acids to diagnose disease, guide therapy, and evaluate susceptibility to the disease before the disease is evident. In a molecular pathology section of a clinical laboratory, it is possible to detect the nucleic acid of a specific organism in any specimen by using molecular biology techniques and diagnose viral diseases such as COVID-19, AIDS, viral hepatitis, etc. Similarly, bacterial and fungal infections can be determined in a short duration by molecular biology techniques. Various types of cancers also could be diagnosed and related therapy could be determined based on molecular biology techniques.

In bacteriological studies, culture methods are lengthy, and most of the methods based on ELISA techniques are indirect in detecting causative organisms. However, tests based on molecular biology are fast, sensitive, specific, accurate, and precise. It is also possible to determine the viral load in certain infections, such as HIV, for effective treatment and reliable diagnosis of certain diseases, such as tuberculosis can be made very fast compared to the culture techniques. Various types of genetic disorders such as sickle cell anemia, cystic fibrosis, muscular dystrophy, etc., can be detected by cytogenetic studies based on molecular biology techniques. By molecular pathology techniques, it is possible to determine the sequence of the human genome.

IMPORTANCE OF MOLECULAR PATHOLOGY TECHNIQUES

BI7.4: Describe applications of molecular technologies like recombinant DNA technology, and PCR in the diagnosis and treatment of diseases with genetic basis

BLOT TECHNIQUES

SAQ: What are blot techniques?

Ans: Blot techniques involve electrophoretic separation of DNA, RNA, or a specific microbial protein in a specimen. Electrophoretically separated patterns of specimen material are then transferred to nitrocellulose or diazobenzyloxymethyl paper (DBM). It is possible to preserve the fractionated electrophoretic pattern. By using techniques such as autoradiography and ELISA, the separated pattern can be visualized, and the specific DNA or RNA fragments can be identified.

SAQ: Write four uses of blot techniques in diagnostic technology.

Ans: The following are the uses of blot techniques:

1. Genetic disorders like sickle cell anemia, beta-thalassemia, etc.
2. Specific viral diseases such as HIV, viral hepatitis
3. Specific cancer such as leukemia and
4. Blot techniques are useful in the preparation of specific diagnostic probes.

SAQ: What is the southern blot technique?

Ans: Southern blot techniques involve fractionation of DNA by electrophoresis and then the transfer of the separated patterns of DNA to a solid support such as a nitrocellulose sheet; followed by subsequent hybridization with a known fractionated labeled DNA for the identification of the gene under test.

SAQ: What is the northern blot technique?

Ans: Northern blot techniques involve fractionation of RNA by electrophoresis and then the transfer of the separated patterns of RNA to a solid support such as a nitrocellulose sheet, followed by subsequent hybridization with a known labeled RNA fraction for the identification of the gene under test.

SAQ: What is western blotting?

Ans: In western blotting specific proteins related to a microorganism, antibodies, or cancer are subjected to electrophoresis and later on, after fractionation, transferred to a solid support such as nitrocellulose paper, followed by subsequent hybridization with a known labeled RNA fraction for the identification of the gene protein under test.

POLYMERASE CHAIN REACTION (PCR)

BAQ: What is polymerase chain reaction (PCR) technology and its importance?

Ans: PCR technology generates sufficient quantities of DNA by increasing (amplifying) the number of copies of the target region of DNA (the template, which serves as the pattern for making copies) without altering the template nucleotide sequence. At the end of a PCR experiment, over a million copies of the original DNA of interest can be obtained as complementary or copy DNA (cDNA). Therefore, PCR provides an efficient method to obtain a sufficient quantity of target DNA required for the identification of specific diseases and genes. The following are various advantages of PCR tests:

1. PCR can identify organisms that cause infectious diseases related to viruses, bacteria fungi, and parasites.
2. Tissue biopsy specimens can be analyzed by PCR for gene mutations that signify the early development of cancer.
3. PCR can detect genetic mutations associated with diseases such as Type 1 and Type 2 diabetes mellitus, maturity-onset diabetes mellitus (MODM), cardiovascular disease, etc.
4. Detection of gene mutations by PCR in preneoplastic disorders, such as inflammatory bowel disease, may be helpful in the early detection of colon cancer.

5. In the case of breast cancer, early detection of mutated genes can precede the histopathologic observation of malignant cells in a breast mass biopsy.
6. Mitochondrial DNA extracted from saliva residue on stamps, shed hair, bone, platelets, and leukocytes have been useful in both diagnostic and forensic pathology.
7. The PCR cDNA can be converted to a probe to detect target DNA or its mRNA transcript using Southern and Northern blot techniques.

Q: Write four advantages of PCR tests.

Ans:

1. PCR can identify organisms that cause infectious diseases related to viruses, bacteria, and fungi.
2. Tissue biopsy specimens can be analyzed by PCR for gene mutations that signify the early development of cancer.
3. PCR can also detect genetic mutations associated with diseases such as Type 1 and Type 2 diabetes mellitus, maturity-onset diabetes mellitus (MODM), cardiovascular disease, breast cancer, colon cancer, etc.
4. Mitochondrial DNA extracted from saliva residue on stamps, shed hair, bone, platelets, and leukocytes have been useful in both diagnostic and forensic pathology.

SAQ: Enumerate sources for PCR test.

Ans: The sources for the PCR test are as follows:

1. The double-stranded DNA (dsDNA) containing the nucleotide sequence of interest is extracted from the sample.
2. Single-stranded DNA (ssDNA) obtained from the dsDNA, which is denatured by breaking the hydrogen bonds between the complementary base pairs adenine-thymine (A-T) and guanine-cytosine (G-C)
3. The target sequence within the ssDNA, which is then amplified by PCR
4. The various samples used for PCR are blood, serum, semen (for forensic purposes), cells grown in culture (for research), and tissue biopsy (from either formalin-fixed paraffin–embedded tissues or frozen tissue sections).

BAQ: Enumerate components for PCR test.

Ans: The components of PCR are as follows:

1. The double-stranded DNA (dsDNA) of interest (separated from the sample).
2. **The primers:** Two primers, known as antisense and sense primers, are required. A primer is typically a sequence in the range of 15–25 nucleotides, whose sequence is complementary to the known nucleotide sequence of interest. Each primer sequence is specific for each unique gene sequence.
3. **Thermus aquaticus (Taq):** Since PCR is performed at high temperatures (55°–94°C), eucaryotic DNA polymerase that functions at 37°C would be degraded. Thermus aquaticus (Taq), which thrives at an optimal temperature of 90°C in hot springs, provides the main source of thermostable DNA. DNA polymerase is cloned from *Pyrococcus furiosus* (Pfu), an organism that grows optimally in geothermal marine sediments at 100°C.
4. **The buffer systems and dNTPs:** The optimal PCR conditions are significantly influenced by the $MgCl_2$ concentration of the buffer system. The four dNTPs are added to the reaction mixture at a concentration of 50–200 nanomoles to provide the source of nucleotides used to extend the primer in the reaction catalyzed by the thermostable DNA polymerase.

Refer to Chapter 19 for the the PCR method and procedure.

GENE CLONING

SAQ: What is gene cloning?

Ans: Gene cloning means the separation of a specific gene from a donor cell and attaching it to a small carrier molecule called a vector and then the introduction of the recombinant gene into a host bacterial cell like *E. coli* and getting multiple copies of the specific gene using bacterial cell culture.

Applications of DNA (RNA) Probes

LAQ: What are DNA and RNA probes? Describe four applications of DNA probes.

Ans: DNA or RNA probe is a single-stranded sequence of DNA or RNA, labeled with radioactive or non-radioactive material and used to determine its complementary sequence in a sample genome present in a specimen like blood, serum, semen, or specific tissue.

DNA probes are first labeled with biotin by attaching it to the base thymine in the nucleotide sequence. The RNA probes can be similarly labeled with biotin through the base uracil.

Applications of DNA (RNA) probes:

1. **Use in diagnostic microbiology:** Traditional microbiological methods employ staining techniques, direct visualization, culturing, and isolation of microorganisms or detection of specific antibodies. However, many organisms are not easily identified by staining. The culture technique is laborious and requires long incubation periods. Some microorganisms require expensive media for culture, and some cannot be cultured. Measuring the host antibody response may not be informative during the acute phase of the infection. It may not distinguish between past and present infections.

   Nucleic acid probes directly detect genetic information of the pathogen in the specimen. Nucleic acid is a relatively stable component, hence specimen viability and transport time may no longer be important issues. With sufficiently sensitive assays, latent viral infections can be detected. Probes can also be designed to directly detect antibiotic resistance genes.

   Infections caused by bacteria such as *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Neisseria gonorrheae* and infections caused by viruses such as herpes simplex types I and II, papillomavirus, adenovirus, Epstein-Barr virus, hepatitis B virus, and AIDS-virus can be detected by the use of appropriate DNA/RNA probes.
2. **Use in the detection of malignancies:** Lymphomas and leukemias are malignancies of the hematopoietic cells. They are conventionally diagnosed based on cell surface proteins and their morphology. Monoclonal antibodies and flow cytometry can usually characterize myeloid and lymphoid malignancies according to the combination of markers present on the cell surface. This information is extremely useful in diagnosis, prognosis, and treatment. In some cases, however, surface markers are not expressed. Sometimes the abnormal population is too small to be detected. DNA probe methodology and nucleic acid hybridization assays may be useful to provide the needed information.

   Some kinds of lymphoma and leukemia have already been characterized by microscopic changes in the DNA of malignant cells. Abnormal chromosomal breakage and exchange of large portions of chromosomes are found in hemopoietic malignancies such as chronic myelogenous leukemia (CML). By using DNA probes, it is possible to detect these various chromosomal aberrations and related diseases.
3. **Use in the detection of genetic diseases:** DNA probe analysis provides an effective method for studying and diagnosing genetic diseases, primarily when the disease is caused by mutations within a single gene. The involved genes can be identified, cloned, and sequenced in normal and abnormal presentations. Genetic diseases such as sickle cell anemia, beta-thalassemia, cystic fibrosis, and familial hypercholesterolemia can be diagnosed with the help of appropriate DNA probes.
4. **Use in HLA-antigen typing:** Human leukocyte antigens (HLA antigens) are proteins derived from loci within the human major histocompatibility complex that are expressed on the surface of some cells. HLA antigen matching between donor and recipient is extremely important in organ and bone marrow transplantation.

HLA antigen mismatch can result in graft rejection in organ transplantation and graft versus host disease in bone marrow transplantation.

Class I HLA antigens include HLA-A, HLA-B and HLA-C antigens. Class II HLA antigens are produced from the D region of the human major histocompatibility complex. Although serological and cell culture methods are used to detect class I and II HLA antigens, D region typing is especially laborious and time-consuming. PCR assays are found to be suitable for the D-region and subregion (DR, DQ and DP) gene typing. Oligonucleotide probes have been devised to detect DR-, DQ-, and DP-region alleles that vary as little as a single base pair.

SAQ: Enumerate four uses of DNA probes.

1. Diagnosis of infections caused by bacteria such as *Mycobacterium tuberculosis*, *Mycobacterium avium, Neisseria gonorrhea*, etc.
2. Diagnosis of infections caused by viruses such as COVID-19, herpes simplex types I and II, papillomavirus, adenovirus, Epstein-Barr virus, hepatitis B virus, and AIDS virus, etc.
3. In the detection of an inherited disease by analysis of fetal DNA, usually with a view to selective termination of pregnancy using an examination of amniotic fluid cells obtained by amniocentesis at 8–18 weeks of gestation, or chorionic villus sampling (CVS), which is carried out from 8 to 10 weeks.
4. Use in HLA-antigen typing.

RECOMBINANT DNA TECHNOLOGY

SAQ: What are restriction enzymes and their applications in gene cloning?

Ans: The restriction enzymes are widespread in bacteria, in which they serve defensive functions by cleaving molecules of foreign DNA. The usefulness of these enzymes is derived from the fact that they do not cleave DNA at random, but recognize and cut specific nucleotide sequences. Examples of restriction enzymes are, Bam HI, EcoRI, HindIII, HeIII, etc.

The enzyme He III cuts DNA in such a way that "blunt ends" are produced, while EcoRI cut DNA asymmetrically so that 'sticky ends' are left; which are extremely useful for reannealing fragments by using enzymes ligases to produce recombinant DNA.

SAQ: Name four enzymes used in the gene cloning procedure.

1. Bam HI, 2. EcoRI , 3. HindIII, 4. HeIII

SAQ: What are recombinant DNA technology and its advantages?

Ans: Recombinant DNA technology involves the modification of a specific DNA by removing the defective gene and combining it with a normal specific gene from another source and introducing modified DNA into a plasmid. The modified plasmid is then introduced as a vector in a bacterial cell such as *E. coli*. Growth of the bacteria in a specific culture medium gives multiple copies of recombinant DNA, which can be used for appropriate gene therapy.

LAQ: Describe the general procedure of recombinant DNA technology.

Ans:

1. Defective DNA molecule under test is fragmented by using enzymes restriction endonucleases. The single-stranded fragments are separated on polyacrylamide gel by electrophoresis.
2. Sequencing of DNA is performed by using the chain terminating method of F. Sanger (or the chemical cleavage method of A. Maxam and W. Gilbert).
3. The separated pattern of DNA on a polyacrylamide gel can be transferred to a piece of nitrocellulose paper using the Southern blot technique. Nitrocellulose paper is treated with a radioactive DNA molecule acting as a probe, to discover the separated pattern.

4. After confirming the base sequence, the DNA fragment of interest is introduced into a plasmid by cutting it using restriction endonucleases. The DNA fragments with the complementary sticky ends of the plasmid are annealed by using the enzyme DNA ligase (Fig. 15.12).

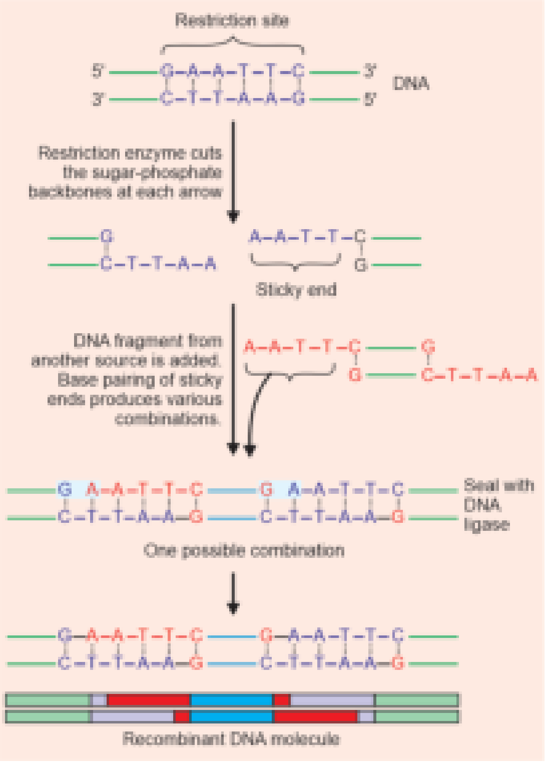

Restriction site
5'
G–A–A–T–T–C
3'
3'
C–T–T–A–A–G
5'
DNA
Restriction enzyme cuts the sugar-phosphate backbones at each arrow
G
C–T–T–A–A
A–A–T–T–C
G
Sticky end
DNA fragment from another source is added. Base pairing of sticky ends produces various combinations.
A–A–T–T–C
G
G
C–T–T–A–A
G A–A–T–T–C G A–A–T–T–C
C–T–T–A–A–G C–T–T–A–A–G
Seal with DNA ligase
One possible combination
G–A–A–T–T–C G–A–A–T–T–C
C–T–T–A–A–G C–T–T–A–A–G
Recombinant DNA molecule

Fig. 15.12: Recombinant DNA technology

5. This recombinant plasmid is then inserted into a bacteria such as *E. coli* and allowed to replicate under appropriate growth conditions in a specific media.
6. The replicated clones of the DNA within the bacterial phase are subsequently recovered by harvesting and lysing the cells (Fig. 15.13).

GENE THERAPY

LAQ: Write a note on gene therapy.

Ans: Gene therapy (Fig. 15.14) is a technique that is used to modify defective genes of a person to treat or cure a specific disease caused by inherited or acquired diseases. Gene therapies can replace a disease-causing gene with a healthy version of the gene or by inactivating a disease-causing gene that is functioning abnormally. The ultimate goal of gene therapy is in the management of inherited as well as acquired diseases to eliminate the underlying biochemical defects, rather than a symptomatic treatment.

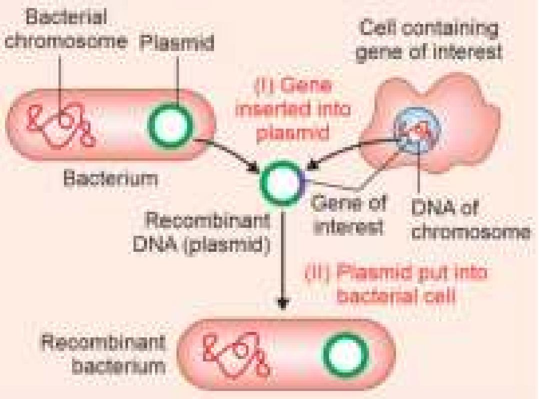

Bacterial chromosome
Plasmid
Cell containing gene of interest
(I) Gene inserted into plasmid
Bacterium
Recombinant DNA (plasmid)
Gene of interest
DNA of chromosome
(II) Plasmid put into bacterial cell
Recombinant bacterium

Fig. 15.13: Introduction of modified DNA in a bacteria

Genetic therapies will be useful to treat many life-threatening diseases. However, they are still under study to decrease potential risks that could include certain types of allergic reactions, or damage to organs or tissues. Recent advances have made genetic therapies much safer and the Food and Drug Administration in the USA has approved some gene transfer therapies for clinical use.

The following are three types of gene therapies: (1) *Ex-vivo* gene therapy, (2) *in-vivo* gene therapy, and (3) *in situ* gene therapy.

1. In *ex-vivo* gene therapy the genetic modification of defective DNA is carried out by recombinant technology outside of the body to produce a normal gene which is introduced back into the patient using a suitable viral vector or a liposome molecule to replace a defective gene.
2. In case of *in-vivo* gene therapy, the direct delivery of a normal gene is performed

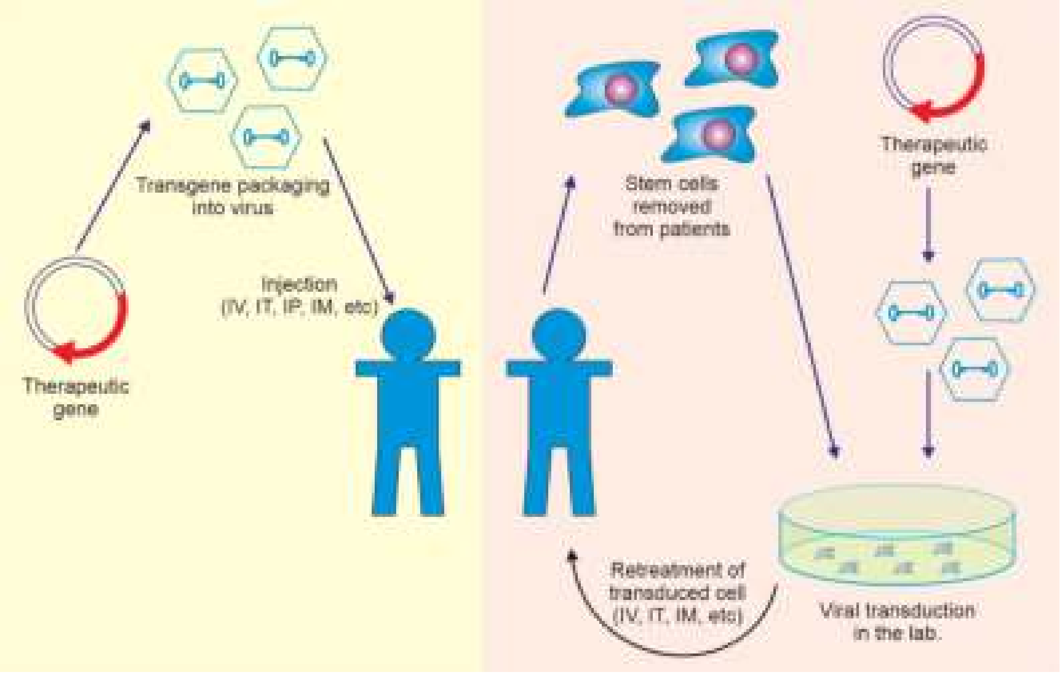

Transgene packaging into virus
Injection (IV, IT, IP, IM, etc)
Therapeutic gene
Stem cells removed from patients
Therapeutic gene
Retreatment of transduced cell (IV, IT, IM, etc)
Viral transduction in the lab.

Fig. 15.14: *In-vivo* (left) and *ex-vivo* (right) gene therapy

either intravenously or locally to a specific organ to replace a defective gene. The normal gene is tagged with a viral vector.

3. *In situ* gene therapy consists of the administration of a normal gene product to a specific site using a viral vector or liposome to replace a defective gene.

There are two basic methods of gene therapy that includes: (1) Germline therapy and (2) Somatic gene therapy.

1. Germline gene therapy is performed by the insertion of normal DNA into the reproductive cells such as eggs or sperm in the human body. Germline gene therapy is useful to correct the genetic variants of the reproductive cells of an individual, and this would also pass down to future generations.
2. Somatic cell gene therapy is performed by the placement of a normal human gene into somatic cells, to replace a defective gene. Somatic cell gene therapy may cure a disease of the patient. However, descendants of the patient do not get the advantage of somatic gene therapy.

Some patients suffering from beta-thalassemia in the USA were treated successfully recently by replacement of the defective gene in bone marrow with a normal gene using a suitable vector.

In the future, gene therapies may be used to prevent, treat, or cure certain inherited disorders, such as hemophilia, sickle cell anemia, cystic fibrosis, alpha-1 antitrypsin deficiency, beta thalassemia, etc. Gene therapies may also be used to treat specific types of cancers or infections such as HIV.

SAQ: Give an example of research on the treatment of Type 1 diabetes mellitus by gene therapy.

Ans: In most of the cases of type 1 diabetes mellitus, beta cells of the pancreas fail to secrete insulin. Proinsulin mRNA can be isolated from pancreatic beta cells. It is attached to polysomes. The polysomes are precipitated by using their specific antibodies. Separated mRNA can be fractionated by using the enzyme-restriction endonucleases and the base sequence in the fractions can be identified by Southern blot technique. When a fraction containing the desired mRNA has been identified, it is used to direct the synthesis of DNA molecules (cDNA) complementary to all of the mRNAs in that fraction. This cDNA can be used as a probe, which can direct the synthesis of insulin when introduced in pancreatic beta cells.

SAQ: Give information on DNA sequencing and equipment used in DNA sequencing.

Ans: DNA sequencing is used to determine the sequence of bases in individual genes, clusters of genes present in operon systems, full chromosomes, or entire genomes of any organism.

A DNA sequencer is an automatic instrument used to determine the DNA sequencing process. A DNA sequencer is used to determine the order of the four bases: Guanine (G), Cytosine (C), Adenine (A), and thymine (T). Genetic defects could be diagnosed by DNA sequencing.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

LAQ: Write a note on restriction fragment length polymorphism (RFLP) based assays.

Ans: Restriction fragment length polymorphism (RFLP) technique (Fig. 15.15) is used to find out the presence of polymorphism in a specific family member. A gene is said to be polymorphic if more than one allele occupies that gene's locus within a population. Gene polymorphisms can occur in any region of the genome. The majority of polymorphisms are silent, i.e. they do not alter the expression of a gene. However, some polymorphic variants of a gene can lead to abnormal expression or to the production of an abnormal form of the protein that may cause a specific disease. For example, substituting phenylalanine with serine at the protein's amino acid position 434 has reduced enzyme activity in metabolizing arachidonic acid to the blood pressure-regulating eicosanoid. A study has shown that humans bearing this variant in one or both of their defective genes have an increased incidence of ischemic stroke, coronary artery disease, and hypertension.

Polymorphisms in DNA closely linked to a disease gene can be used to predict the inheritance of the altered allele. RFLP technique is useful in establishing the linkage of a region of the genome to disease, in family studies of inherited diseases (classical genetics), and in identifying regions of the genome altered during neoplasia (somatic mutations).

The basic steps of RFLP are as follows:

1. Extraction of DNA from individuals in a family A and B.
2. Use of restriction enzymes to cut DNA. SmaI is a restriction endonuclease used to cut DNA at the recognition sequence 5′-CCC/GGG-3′, generating DNA fragments with blunt termini.
3. Electrophoresis of DNA fragments on an agarose gel to separate DNA fragments of A and B.
4. Transfer of the DNA in the gel to a nylon membrane by Southern blot.
5. Identification of polymorphism (Fig. 15.15).

DNA sequencing methodology is a fast and accurate method to find out DNA polymorphism in a family compared to the RELP technique.

BAQ: Write a short note on epigenetics.

Ans: Epigenetics studies genetic effects not encoded in the DNA sequence of an organism. Such effects on cellular and physiological phenotypic traits may result from external or environmental factors that switch genes on and off and affect gene expressions. These alterations may or may not be heritable. The epigenetic changes may last through cell divisions for the duration of life of the cell and may also last for multiple generations even though they do not involve changes in the underlying DNA sequence of the organism.

DNA methylation works by adding a chemical group to DNA. Typically, this group is added to specific places on the DNA, where it blocks the proteins that attach to DNA to "read" the gene. This chemical group can be removed

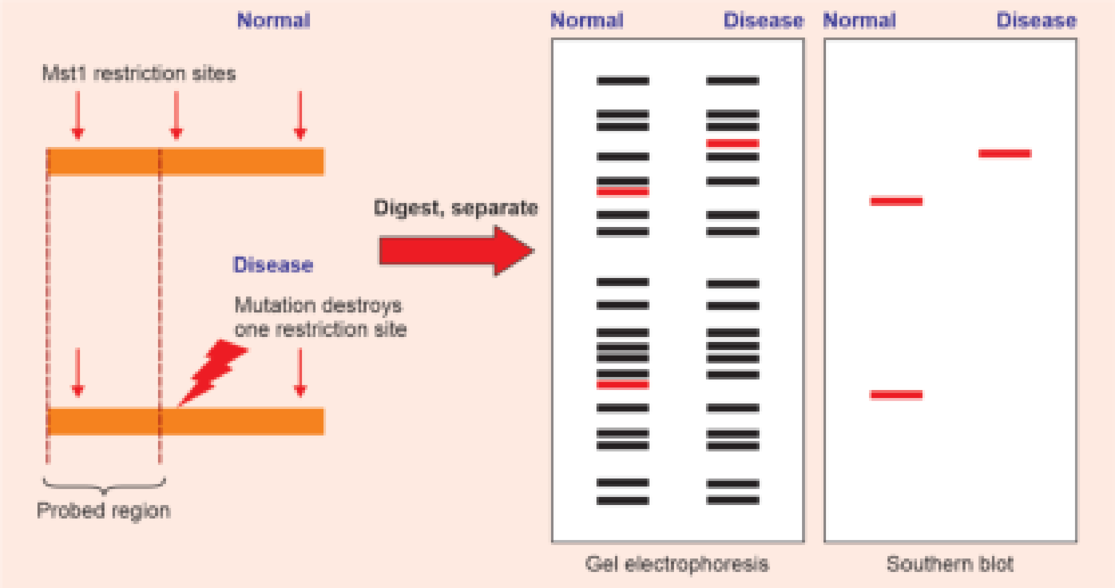


Fig. 15.15: Diagnosis of polymorphism by RFLP technique

through a process called demethylation. Typically, methylation turns genes "off" and demethylation turns genes "on."

DNA wraps around histones. When histones are tightly packed together, proteins that 'read' the gene cannot easily access the DNA, so the gene is turned "off." When histones are loosely packed, more DNA is exposed or not wrapped around a histone and can be accessed by proteins that 'read' the gene, so the gene is turned "on." Chemical groups can be added or removed from histones to make the histones more tightly or loosely packed, turning genes "off" or "on".

Unlike genetic changes, epigenetic changes are reversible and do not change the DNA sequence, but they can change the way a DNA sequence is read.

Epigenetics change throughout life. Epigenetics at birth is not the same as epigenetics during childhood or adulthood.

Not all epigenetic changes are permanent. Some epigenetic changes can be added or removed in response to changes in behavior or environment.

Smoking can result in epigenetic changes. For example, at certain parts of the Aryl Hydrocarbon Hydroxylase Regulator (AHRR) gene, smokers tend to have less DNA methylation than non-smokers. The difference is greater for heavy smokers and long-term smokers. After quitting smoking, former smokers can begin to have increased DNA methylation at this gene.

Some epigenetic changes increase cancer risk. For example, having a mutation in the BRCA1 gene increases the risk of breast cancer. Colorectal cancers have abnormal methylation at DNA regions near certain genes, which affects the expression of these genes.

Environment and lifestyle changes during pregnancy can change the baby's epigenetics. Some of these changes can remain for decades and might make the child more likely to get specific diseases.

SAQ: Write a short note on bioinformatics.

Ans: Bioinformatics is a field that deals with the application of software tools used to capture and interpret biological data. The bioinformatics field makes use of computer science, biomedical engineering, mathematics, and statistics, to analyze and interpret biological data. The main components of bioinformatics are: (1) Creation of databases; (2) Development of algorithms and statistics; and (3) Analysis of data and interpretation of the analyzed data.

Bioinformatics is useful to study the principles behind the organization of nucleic acids, protein molecules, and DNA and RNA sequences, which are useful to understand the genetic basis of diseases, specific genetic adaptations, and differences between various populations. Methods based on bioinformatics are applied in the study of genomics, proteomics, and drug designing by molecular modeling.

Multiple Choice Questions

Q1. Degeneracy of genetic code means

A. Codons code only for specific amino acid
B. No anticodon on the tRNA molecule
C. Multiple codons must decode the same amino acids
D. Specific codon decodes many amino acids

Q2. Genetic code is

A. Overlapping
B. Nonoverlapping
C. Not universal
D. A and C

Q3. mRNA is complementary to the nucleotide sequence of

A. Template strand
B. Ribosomal RNA
C. tRNA
D. Coding strand

Q4. Which is the smallest unit of DNA capable of coding for the synthesis of a polypeptide?

A. Cistron
B. Repressor gene
C. Operon
D. Replicon

Q5. The factor responsible for the termination of transcription is

A. Rho (σ) factor
B. Rho (α) factor
C. α factor
D. β factor

Q6. The function of a repressor protein in an operon system is the prevention of protein synthesis by binding to

A. A specific region of the operon preventing transcription of structural genes
B. The ribosome
C. The DNA polymerase
D. The RNA polymerase

Q7. Pribnow box consists of the sequence

A. 5′-UAUCAA-3′
B. 5′-GAGCCA-3′
C. 5′-TATAAT-3′
D. 5′-TACTAG-3′

Q8. 5′-Terminus of mRNA molecule is capped with

A. Adenosine triphosphate
B. 7-Methylguano-sine triphosphate
C. Guanosine triphosphate
D. Guanosine diphosphate

Q9. Which one is the protein chain initiating codon?

A. UUU
B. GGU
C. AUG
D. AAA

Q10. AUG codes for

A. Phenylalanine
B. Arginine
C. Leucine
D. Methionine

Q11. In the biosynthesis of proteins the chain-terminating codons are:

A. UAA
B. UAG
C. UGA
D. All of the above

Q12. Initiation of translation requires these factors

A. EIFs
B. IF-2
C. Rho (σ) factor
D. IF-I
E. B and D

Q13. In the case of eukaryotes, initiation of protein synthesis begins with the binding of this ribosomal unit with mRNA

A. 60S
B. 40S
C. 30S
D. 70S

Q14. Initiation of protein synthesis requires

A. ADP
B. ATP
C. GTP
D. GDP

Q15. The function of the enzyme aminoacyl-tRNA synthetase is

A. Dissociation of discharged tRNA from 80S ribosome
B. Termination of protein synthesis
C. Charging of tRNA with specific amino acids
D. A and B

Q16. Translation results in the formation of

A. Amino acid
B. Protein
C. mRNA
D. rRNA

Q17. The mushroom poison amanitin is an inhibitor of

A. Protein synthesis
B. DNA synthesis
C. RNA polymerase II
D. A and C

Q18. The antibiotic tetracycline prevents the synthesis of polypeptides by

A. Blocking mRNA formation from DNA
B. Blocking of release of peptides from ribosomes
C. Preventing binding of aminoacyl tRNA to A site of the ribosome
D. Competing with mRNA for ribosomal binding sites

Q19. The antibiotic chloramphenicol prevents protein synthesis by

A. Binding to 50S ribosome and inhibiting peptidyl transferase
B. Blocking mRNA formation
C. Binding with RNA polymerase II
D. B and C

Q20. The antibiotic streptomycin prevents the synthesis of polypeptides by

A. Inhibiting peptidyl transferase
B. Inhibiting DNA polymerase
C. Inhibiting initiation process
D. Inhibiting translocation

Q21. The antibiotic erythromycin inhibits

A. Peptidyl transferase activity
B. Translocation
C. RNA polymerase II
D. Binding of aminoacyl tRNA

Q22. The binding of prokaryotic DNA-dependent RNA polymerase to promoter sites of genes is inhibited by the antibiotic

A. Tetracycline
B. Streptomycin
C. Terramycin
D. Rifamycin

Q23. Novobiocin antibiotic inhibits

A. tRNA
B. mRNA
C. DNA replication
D. rRNA

Q24. Ciprofloxacin antibiotic inhibits the replication, transcription, and repair of

A. rRNA
B. mRNA
C. DNA
D. tRNA

Q25. The antibiotic ciprofloxacin inhibits

A. DNA topoisomerase II and IV
B. DNA gyrase
C. DNA polymerase III
D. DNA polymerase I

Q26. The antibiotic rifampicin inhibits

A. Unwinding of DNA
B. Initiation of transcription
C. Initiation of translation
D. Initiation of replication

Q27. Actinomycin D

A. Blocks the movement of RNA polymerase
B. Binds double-stranded DNA
C. Binds single stranded RNA
D. A and B

Q28. The structural genes of the Lac operon operate only when this is available

A. Glucose
B. Lactose
C. Fructose
D. Galactose

Q29. This region should be free on the Lac operon to start structural gene transcription

A. Promoter site
B. Y gene
C. Operator locus
D. A gene

Q30. In the lac operon concept, this is recognized by a (an)

A. Repressor
B. Inducer
C. Promoter
D. Operator

Q31. Components of Lac operon

A. A regulatory gene
B. An operator
C. A promoter
D. All of the above

Q32. The regulatory I gene of the lac operon

A. Is inhibited by lactose
B. Is responsible to synthesize lactose
C. Forms a regulatory protein
D. Is inhibited by the repressor protein

Q33. RNA polymerase binds to the lac operon at the following site

A. Promoter region
B. Z gene
C. Operator locus
D. Regulatory I gene

Q34. Transcription of Z, Y, and A genes of the lac operon is prevented by

A. Lactose
B. Promoter
C. Inducer
D. Repressor

Q35. Transcription of structural genes of the lac operon is prevented by binding of the repressor tetramer to

A. Operator locus
B. Regulatory I gene
C. Promoter
D. Z gene

Q36. Lactose or its analogs act as positive regulators of lac operon by

A. Attaching to the I gene and preventing its expression
B. Binding to repressor subunits so that the repressor cannot attach to the operator locus
C. Increasing the synthesis of catabolite gene activator protein
D. Attaching to the promoter region and facilitating the binding of RNA polymerase holoenzyme

Q37. Expression of structural genes of lac operon depend on all of the following *except*

A. Presence of lactose
B. Repressor tetramer
C. Presence of glucose
D. ATP

Q38. The coding sequences in the lac operon include

A. I gene
B. I gene, operator locus, and promoter
C. Z, Y and A genes
D. I, Z, Y and A genes

Q39. The function of the enzyme DNA ligase is to

A. Synthesize RNA primers
B. Connect the end of two DNA chains
C. Unwind the double helix
D. Introduce superhelical twists

Q40. Restriction endonucleases

A. Cut RNA chains at specific locations
B. Excise introns from hnRNA
C. Remove Okazaki fragments
D. Act as defensive enzymes to protect the host bacterial DNA from the DNA of foreign organisms

Q41. Lethal mutation may lead to

A. Clinical condition like muscular dystrophy
B. Clinical condition like thalassemia
C. Death of the organism
D. Clinical condition like hemophilia

Q42. Restriction endonucleases recognize and cut a certain sequence of

A. Single-stranded DNA
B. Double-stranded DNA
C. RNA
D. Protein

Q43. Repressor binds to which DNA sequence and regulates the transcription?

A. Attenuator B. Terminator
C. Operator D. Inducer

Q44. Okazaki fragment is related to

A. sRNA synthesis B. Protein synthesis
C. DNA synthesis D. tRNA synthesis

Q45. TATA BOX is the site for the binding of

A. DNA polymerase
B. DNA-dependent RNA polymerase
C. DNA topoisomerase
D. Polynucleotide phosphorylase

Q46. Reverse transcriptase is capable of synthesizing

A. RNA from DNA B. DNA from RNA
C. tRNA from RNA D. cDNA from RNA

Q47. Ultraviolet light may damage a DNA strand causing

A. Two adjacent pyrimidine residues form covalently bonded dimer
B. Disruption of phosphodiesterase linkage
C. Two adjacent purine residues form the covalently bonded dimer
D. Disruption of noncovalent linkage

Q48. Replication of DNA is

A. Conservative B. Nonconservative
C. Semiconservative D. A or B

Q49. Which of the following may lead to mutations?

A. Ionizing radiations B. Alkylating agents
C. UV radiations D. A, B and C

Q50. Exposure of DNA to ultraviolet radiation can lead to the formation of

A. Guanine dimers B. Adenine dimers
C. Uracil dimers D. Thymine dimers

Q51. Damage to DNA caused by ultraviolet radiation can be repaired by all these *except*:

A. DNA ligase
B. UVR ABC exonuclease
C. DNA polymerase I
D. A and C

Q52. Xeroderma pigmentosum results from a defect in

A. DNA ligase
B. UvrABC exonuclease
C. DNA polymerase I
D. DNA ligase

Q53. All the following statements about xeroderma pigmentosum are true *except*

A. Its inheritance is autosomal dominant
B. Its inheritance is autosomal recessive
C. UvrABC exonuclease is defective
D. It results in multiple skin cancers

Q54. Substitution of an adenine base by guanine in DNA is known as

A. Transversion
B. Frameshift mutation
C. Transposition
D. Transition

Q55. Substitution of a thymine base by adenine in DNA is known as

A. Transversion
B. Frameshift mutation
C. Transposition
D. Transition

Q56. A point mutation results from

A. Deletion of a base
B. Insertion of a base
C. Substitution of a base
D. All of these

Q57. Substitution of a base can result in a

A. Mis-sense mutation
B. Nonsense mutation
C. Silent mutation
D. All of the above

Q58. A silent mutation is most likely to result from

A. Substitution of the third base of a codon
B. Substitution of the first base of a codon
C. Conversion of a nonsense codon into a sense codon
D. Conversion of a sense codon into a nonsense codon

Q59. Amino acid sequence of the encoded protein is not changed in

A. Mis-sense mutation
B. Silent mutation
C. Nonsense mutation
D. A and C

Q60. Suppressor mutations occur in

A. Promoter regions
B. Anticodons of tRNA
C. Silencer elements
D. Structural genes

Q61. Suppressor tRNAs can neutralize the effects of mutations in

A. Enhancer elements
B. Promoter regions
C. Structural genes
D. All of these

Q62. Mutations in promoter regions of genes can cause

A. Change in the reading frame of downstream structural gene
B. Poor transcription
C. Premature termination of translation
D. All of the above

Q63. Mitochondrial protein synthesis is inhibited by

A. Tetracyclines
B. Sulfa drugs
C. Chloramphenicol
D. None of these

Q64. Endonucleases

A. Can cleave peptide linkage
B. Act on lipids
C. Can act on the secondary structure of the protein
D. Cleave phosphodiester bond within a polynucleotide chain

Q65. A plasmid is a

A. Single-stranded DNA
B. Double-stranded circular DNA
C. Double-stranded linear DNA
D. A or C

Q66. DNA fragments can be identified by

A. Western blotting
B. Southern blotting
C. Northern blotting
D. A and C

Q67. RNA in a mixture can be identified by

A. Southern blotting
B. Western blotting
C. Northern blotting
D. All of the above

Q68. HIV protein in blood can be detected by

A. Southern blotting
B. Northern blotting
C. Western blotting
D. A and B

Q69. Monoclonal antibodies are prepared by cloning

A. Hybridoma cells
B. Macrophages
C. Myeloma cells
D. B-Lymphocytes

Q70. The DNA polymerase commonly used in the polymerase chain reaction is obtained from

A. *E. coli*
B. Yeast
C. Adenoviruses
D. *T. aquaticus*

Q71. Optimum temperature of DNA polymerase of *T. aquaticus* is

A. 37°C B. 72°C
C. 54°C D. 30°C

Q72. Transgenic animals may be prepared by introducing a foreign gene into

A. Somatic cells of young animals
B. Fertilized egg and implanting the egg into a foster mother
C. Testes and ovaries of animals
D. A viral vector and infecting the animals with the viral vector

Answers

1. C **2.** B **3.** D **4.** A **5.** A **6.** A **7.** C **8.** B **9.** C **10.** D
11. D **12.** A **13.** B **14.** C **15.** C **16.** B **17.** C **18.** C **19.** A **20.** C
21. B **22.** D **23.** C **24.** C **25.** A **26.** B **27.** D **28.** B **29.** C **30.** A
31. D **32.** C **33.** A **34.** D **35.** C **36.** B **37.** D **38.** C **39.** B **40.** B
41. C **42.** B **43.** C **44.** C **45.** B **46.** D **47.** A **48.** C **49.** D **50.** D
51. C **52.** B **53.** A **54.** D **55.** A **56.** C **57.** D **58.** A **59.** B **60.** B
61. C **62.** B **63.** C **64.** D **65.** B **66.** B **67.** C **68.** C **69.** C **70.** D
71. B **72.** D

Chapter

16

Cancer and Tumor Markers

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

The Greek term carcinoma is the medical term for a malignant tumor, derived from epithelial cells. It is Celsus, who translated *carcinos* into the Latin term cancer, meaning crab. Galen used "oncos" to describe all tumors. It is the root of the word oncology.

Cancer is the most common cause of death after cardiovascular disease: Humans of all ages develop cancer. The incidences of many cancers increase with age. The clinical signs and symptoms in patients suffering from cancer are directly related to a tumor in most cases. The tumor may cause obstruction of ducts, exert pressure on nerves, or may destroy normal tissue.

A large number of annual worldwide deaths are caused by malignant neoplasms. Malignant neoplasm is the medical term for 'cancer'. It is a class of disease in which a group of cells displays uncontrolled growth, invasion, and sometimes metastasis (spread to other locations in the body via lymph or blood). Most cancers form a tumor but some cancers, like leukemia, do not form a tumor.

Definitive diagnosis of cancer requires the histologic examination of a biopsy specimen, although the initial indication of malignancy can be symptomatic or abnormalities are seen by radiographic imaging. Most cancers can be treated, and some are forced into remission, depending on the specific type, location, and stage.

Once diagnosed, cancer is usually treated with a combination of surgery, chemotherapy, and radiotherapy. Treatments are becoming more specific for different varieties of cancer with advanced research work in oncology. Specific drugs have been developed that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. The prognosis of cancer patients is influenced mainly by the type of cancer, as well as the stage, or extent of the disease. In addition, histopathological examinations and the presence of specific tumor markers can also be useful in establishing prognosis, as well as in determining individual treatments.

Competency achievement: The student should be able to:

IM13.1: Describe the clinical epidemiology and inherited and modifiable risk factors for common malignancies in India

BAQ: Define cancer and metastasis. What are the main characteristic features of cancer?

Ans: Cancer is a term used for diseases in which abnormal cells divide without control and can invade nearby tissues.

Metastasis means the spread of cancer cells from the first place where formed to another part or parts of the body.

Cancer cells are characterized by the following main three properties:

1. Diminished or unrestrained control of growth
2. Invasion of local tissue and
3. Spread, or metastasis, to other parts of the body.

BAQ: Define the following terms related to cancer: Carcinogens, oncology, neoplasm, benign tumor, malignant tumor, sarcoma, lymphoma, and lipoma.

Ans:

1. Carcinogen is any substance that causes cancer.
2. Oncology is the science that deals with the study of tumors.
3. Neoplasia means new growth and uncontrolled growth of cells which results in tumors.
4. Benign tumor is a type of tumor that shows diminished control of growth and does not invade local tissue or spread to other parts of the body.
5. Malignant tumor is formed by the uncontrolled proliferation of cells and the spread of cells to various parts of the body by metastasis.
6. Sarcoma is a type of cancer that initiates in the bone or the soft tissues of the body.
7. Lymphoma is a group of blood and lymph tumors that develop from lymphocytes.
8. A lipoma is a slow-growing, fatty lump. It is often situated between the skin and the underlying muscle layer. A lipoma is harmless and not cancerous. Treatment generally is not required.

THE CARCINOGENS

BAQ: Write a note on chemical carcinogens.

Ans: Almost 80% of human cancers are caused by chemical carcinogens in nature. Entry of the chemical into the body may occur by one of the following mechanisms:

1. **Lifestyle (cigarette smoking):** Cigarette contains many carcinogens. Benzopyrenes are an important group of carcinogens present in cigarettes along with other deleterious substances such as nicotine, carbon monoxide, nitrogen dioxide, etc.
2. **Drugs:** Therapeutic drugs such as diethylstilbestrol.
3. **Diet:** Contaminated foodstuff by fungus, *Aspergillus flavus* (which produces aflatoxin B).
4. **Occupation (aniline, asbestos, benzene):** These carcinogens can covalently bind to purines, pyrimidines, and phosphodiesterase bonds of DNA, causing irreparable damage, and leading to mutations.
5. **Radiation energy:** X-rays, ultraviolet rays, and γ-rays have been proven mutagenic, causing cancers. Exposure to UV rays results in the formation of pyrimidine dimers in DNA, while X-rays cause the production of free radicals and superoxides which cause breakage in DNA molecules leading to somatic mutations.

SAQ: Enumerate four names of chemical carcinogens.

Ans: Benzopyrenes, diethylstilbestrol, asbestos and aflatoxin B.

PROTO-ONCOGENES AND ONCOGENES

SAQ: What are proto-oncogenes and oncogenes.

Ans: Proto-oncogenes are present in the DNA of a normal cell. Proto-oncogenes play a very important role in the normal growth of the cell by controlling the amount of growth factors and related proteins. Proto-oncogenes also facilitate programmed cell death, in the case of the formation of an abnormal cell. Carcinogens and certain viruses are capable of mutating proto-oncogenes.

A mutated proto-oncogene is then converted to oncogenes. Oncogenes express uncontrolled proliferation of growth factors and related proteins, which leads to the formation of a cancer cell.

ONCOGENIC VIRUSES

BAQ: Write a note on oncogenic viruses.

Ans: Oncogenic viruses are capable of initiating the formation of cancer cells and their proliferation.

The integration of viral genes into the host DNA is a probable etiological factor of carcinogenesis. The virus genes become part of the cellular DNA. When the viral genomes are replicated along with cellular replication, the regulatory checks and balances of the cellular mechanism are lost, leading to uncontrolled multiplication of the cells.

The following are the viruses possibly oncogenic in human beings (Table 16.1):

Table 16.1: Oncogenic viruses

Virus	Abbreviation	Associated human cancer
Hepatitis B virus	HBV	• Hepatoma
Epstein-Barr virus	EBV	• Burkitt's lymphoma (BL) • Nasopharyngeal carcinoma (NPC)
Human papilloma	HPV	• Uterine, cervical
Human T cell	HTLV	• Lymphomas and leukemia virus
Herpes simplex type 1	HSV1	• Oral cancer
Herpes simplex type 2	HSV2	• Cervical carcinoma

SAQ: Write two names of oncogenic viruses.

Ans.

1. Human papilloma (HPV) that causes cervical cancer
2. Epstein-Barr virus (EBV) that causes Burkitt's lymphoma.

BAQ: Write a note on cancer.

Ans: Cancer is a term used for diseases in which abnormal cells divide without control and can invade nearby tissues. Cancer is the most common cause of death after cardiovascular disease. Humans of all ages develop cancer. The incidences of many cancers increase with age. The clinical signs and symptoms in patients suffering from cancer are directly related to a tumor in most cases. The tumor may cause obstruction of ducts, exert pressure on nerves, or may destroy normal tissue.

More than 200 different types of human cancers have been recognized. Carcinomas are cancers arising from epithelial cells, and those arising from connective tissue are known as sarcomas.

About 15 % of annual worldwide deaths are caused by malignant neoplasms. Malignant neoplasm is the medical term for 'cancer'. It is a class of diseases in which a group of cells display uncontrolled growth, invasion, and metastasis. Metastasis means the spread of cancer cells to other locations in the body through lymph or blood. Most cancers form a tumor but some cancers, like leukemia, do not form a tumor.

All cancers are multifactorial in origin. They include genetic, hormonal, metabolic, physical, chemical, and environmental factors. Most human cancers are produced spontaneously without a particular cause. However, all cancers originate usually from one aberrant cell, which goes on to multiply and produces a tumor mass. The aberrant cells are usually destroyed by the immune system. Most cancers are caused by chemical carcinogens, radiation energy, and viruses. These agents may damage DNA or interfere with its replication or repair. Elderly individuals tend to become immunocompromised with advancing age. Hence more than 70% of new cancer cases occur in persons over 60 years.

Definitive diagnosis of cancer requires the histologic examination of a biopsy specimen, although the initial indication of malignancy can be symptomatic or abnormalities are seen by radiographic imaging. Most cancers can be treated, and some are forced into remission, depending on the specific type, location, and stage.

Once diagnosed, cancer is usually treated with a combination of surgery, chemotherapy, and radiotherapy. Treatments are becoming more specific for different varieties of cancer with advanced research work in oncology. Specific drugs have been developed that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. The prognosis of cancer patients is influenced mainly by the type of cancer, as well as the stage, or extent of the disease. In addition, histopathological examinations and the presence of specific tumor markers can also be useful in establishing prognosis, as well as in determining individual treatments.

LAQ: Describe the clinical epidemiology and inherited and modifiable risk factors for common malignancies in India.

Ans: Lung, breast, oral, stomach, and cervical cancer are the most common cancers in India. According to a survey conducted by the Indian Council of Medical Research (ICMR), lung cancer accounts for 9.3 percent of all death cases. Breast cancer is the most frequent cancer in women globally and represents the second leading cause of cancer death among women after lung cancer. Oral cancer is the sixth most common type of cancer globally and India contributes to almost one-third of the total burden. India is the second country with the highest number of oral cancer cases. Gastric cancer is the fifth most common cancer among males and the seventh most common cancer among females in India. Similarly, cervical cancer contributes to approximately 5–25% of all cancers in women.

Lung cancer

Cigarette smoking is the number one risk factor for lung cancer. Lung cancer arises from the cells of the respiratory epithelium. Lung cancer can be divided into two broad categories: Small cell lung cancer (SCLC) is a highly malignant tumor and accounts for 15% of lung cancer cases. Non-small cell lung cancer (NSCLC), which accounts for the remaining 85% of cases.

Cigarette smoking can cause cancer of the mouth, throat, larynx, trachea, esophagus, stomach, colon, rectum, liver, pancreas, renal pelvis, urinary bladder, and cervix, and also may be responsible for initiating acute myeloid leukemia.

Lung cancer: Preventive measures

Primary lung cancer prevention measures include interventions that block the initiation of cancer by quitting smoking and reducing exposure to tobacco smoke.

Secondary lung cancer prevention measures relate to detecting cancer early by undergoing screening imaging tests.

Breast cancers

Genetic predisposition accounts for approximately 5–10% of all breast cancers. Mutations in two autosomal dominant genes, BRCA1 and BRCA2 have been linked to familial breast cancer. India is going through an epidemiologic transition. It is reported that due to epidemiologic transition in India, the incidence of breast cancer is rising rapidly. Epidemiologic transition changes relate to reproductive risk factors, dietary habits, specific genetic factors, and increasing life expectancy.

An increase in breast cancer incidence is due to various modifiable risk factors. In women over 40 years of age, an increase in breast cancer incidences was observed with late-stage of presentation, lack of awareness about screening, examination costs, fear, and stigma associated with the disease are the major factors for early case presentation to physicians.

Introduction of educational strategies as preventive measures for breast cancer:

Educational strategies should be planned keeping in the following points:

1. Modification of the lifestyle
2. Early planning of pregnancy
3. Promoting breastfeeding
4. Increase in physical activity and
5. Reduction in obesity by maintaining body mass index (BMI) > 25.

Colorectal cancer (CRC): The risk of developing colorectal cancer (CRC) is influenced by environmental and genetic factors. An increased number of colonoscopy screenings with the removal of adenomatous polyps in the distal colon could reduce the incidences of CRC.

Oral cancer: Oral cancer is the most common cancer among men in India and the fifth most frequently occurring cancer among women.

Gastric cancer: In India, the incidence rate of gastric cancer is very low compared to that in Western countries.

Modifiable risk factors for oral and gastric cancers: Limiting exposure to the following avoidable risk factors may lower the risk of developing oral cancers and certain other types of cancers such as stomach cancers: Smoking, tobacco, alcohol, cancer-causing chemicals (asbestos, nickel, benzene), and substances (aflatoxins), chronic inflammation, increased fast food intake, hormonal changes, immunosuppression, obesity and exposure to radiations.

Cervical cancer: The most important risk factor for cervical cancer is human papillomavirus (HPV) infection. Other major risk factors are multiple sexual partners and the early onset of sexual activity. Herpes simplex virus type 2 (HSV-2) is transmitted primarily by sexual contact and therefore has been implicated as a risk factor for cervical cancer. Other factors that may contribute to the initiation of cervical cancer are immunodeficiency, smoking, socioeconomic factors, and age.

Preventive measures for cervical cancer:

1. Pap smear examination is recommended for early detection of cervical cancer. In a pap smear test, cells from the surface of the cervix and the area around it are removed and examined under a microscope for the detection of abnormal cells that may lead to cervical cancer.
2. It is necessary to follow the World Health Organization's (WHO) global strategy for cervical cancer elimination (2020): According to the recommended strategy, women globally should be screened regularly for cervical cancer disease and effective treatment for those who are diagnosed with cervical cancer. The administration of human papillomavirus (HPV) vaccines are recommended for female.

DEFINITION AND CHARACTERISTIC FEATURES OF CANCER

Competency achievement: The student should be able to:

BI10.1: Describe the cancer initiation, promotion, oncogenes, and oncogene activation. Also, focus on p53 and apoptosis

LAQ: Define and describe the cancer initiation, promotion, cancer-pathophysiology oncogenes, and oncogene activation. Explain the role of the p53 gene in the prevention of cancer.

Ans: Cancer is a disease in which some of the body cells grow uncontrollably and spread to other parts of the body.

In normal circumstances, the proliferation of body cells is under strict control. The cells differentiate, divide and die sequentially in a healthy organism. The characteristic feature of cancer is loss of control of cellular growth and further development, leading to excessive proliferation and spread of cells.

Cancer cells are characterized by the following three properties:

1. Diminished or unrestrained control of growth
2. Invasion of local tissue and
3. Spread, or metastasis, to other parts of the body.

The tumors are of two types:

1. **Benign tumors:** Usually these tumors grow by expansion and remain encapsulated in a layer of connective tissue. Normally benign tumors show diminished control of growth and do not invade local tissue or spread to other parts of the body.
2. **Malignant tumors or cancer:** These are invariably life-threatening due to uncontrolled proliferation and spread of cells to various parts of the body (metastasis).

Pathophysiology of cancer: In the case of a healthy individual, the normal body has over a trillion cells that divide at a standard rate and pace. When cancer develops, specific normal cells turn into cancer cells. Cancer cells have different DNA than healthy cells.

- The first step in the case of a healthy cell transforming into a cancer cell is the change of the proto-oncogenes to oncogenes. Proto-oncogenes are genes that are coded to maintain normal cell growth. An oncogene is a gene that has changed to make the cells grow and divide faster. In cancer cells, the cell grows and divides very quickly.
- In the second step, the tumor suppressor genes get turned off. Tumor suppressor genes are part of a healthy cell's DNA that helps to stop cancer from forming in healthy cells. Tumor suppressor genes help to decrease cell growth. When these genes are turned off, the cell grows and divides very quickly.
- The last step in the formation of a cancer cell is the failure of the functions of DNA repair genes. DNA repair genes help healthy cells to recognize if something is wrong with their DNA and how to fix it. When these genes get turned off, the cell fails to understand that it is sick, and it is unable to fix any problems with its DNA.

The p53 gene is a tumor suppressor gene. Activated p53 promotes cell cycle arrest to facilitate the DNA repair process or apoptosis, which means programmed cell death, in case an abnormal cell formation takes place. By this means p53 gene expression works to prevent the propagation of cells with serious DNA damage. Loss of the p53 gene or mutation of the p53 gene often leads to the formation progression of cancerous growth.

Mutation of Proto-oncogenes (Fig. 16.1)**:** Proto-oncogenes are present in the DNA of a normal cell. Proto-oncogenes play a very important role in the normal growth of the cell by controlling the amount of growth factors and related proteins. Proto-oncogenes also facilitate programmed cell death, in the case of the formation of an abnormal cell. Carcinogens and certain viruses are capable of mutating proto-oncogenes. A mutated proto-oncogene is then converted to oncogenes, which express uncontrolled proliferation of growth factors and related proteins, which leads to the formation of a cancer cell.

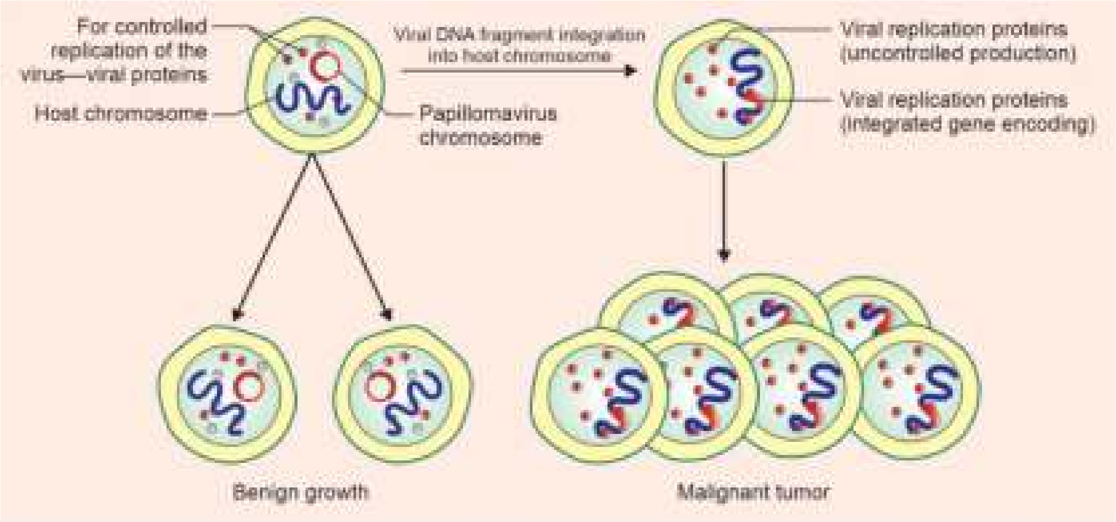

For controlled replication of the virus—viral proteins
Host chromosome
Viral DNA fragment integration into host chromosome
Papillomavirus chromosome
Viral replication proteins (uncontrolled production)
Viral replication proteins (integrated gene encoding)
Benign growth
Malignant tumor

Fig. 16.1: Action of the oncogenic virus on oncogene and formation and activation of oncogene

LAQ: Write a note on metastasis.

Ans: Metastasis is the spread of cancer cells from a primary site of origin to other tissues, where they grow as secondary tumors.

Once a cell becomes a tumor cell, its growth rate increases with an increasing tendency to invade and metastasize, which is the major problem presented by cancer. Metastasis depends on the cancer cells acquiring two separate abilities—increased motility and invasiveness. Progressive tumor growth is angiogenesis-dependent. Tumor cells secrete angiogenic growth factors such as basic or acidic growth factors. These growth factors promote the proliferation of endothelial cells and the formation of new blood capillaries.

Some cancer cells acquire the ability to penetrate the walls of lymphatic and blood vessels. They can circulate through the bloodstream (circulating tumor cells) to other sites and tissues in the body. This process is known as lymphatic or hematogenous spread respectively.

The tumor cells then come to rest at another site, they re-penetrate through the vessel or walls, continue to multiply, and eventually, another clinically detectable tumor is formed. This new tumor is known as a metastatic (or secondary) tumor.

When tumor cells metastasize, the new tumor is called a secondary or metastatic tumor, and its cells are like those in the original tumor. If breast cancer metastasizes to the lungs, the secondary tumor is made up of abnormal breast cells, not abnormal lung cells. The tumor in the lung is then called metastatic breast cancer (and not lung cancer).

The symptoms of metastasis vary with the location of the tumors. Initially, nearby lymph nodes are affected early. The liver, lungs, bones, and brain are the most common metastasis locations for solid tumors. In lymph nodes, a common symptom is lymphadenopathy. Metastasis in the lungs leads to cough, hemoptysis, and dyspnea. Hepatomegaly and jaundice are the symptoms of the affected liver. Similarly, bone pain and an increased tendency to bone fracture are the signs of metastatic bone. When the brain is affected, the neurological symptoms observed are headaches, seizures, and vertigo. Some patients, however, do not show any symptoms.

The following various events may lead to the initiation of cancer:

- **Chromosomal translocation:** Chromosomal translocation usually results in the overexpression of proto-oncogenes. Some of the tumors exhibit chromosomal abnormalities due to the rearrangement of genetic material by splitting off a small fragment of a chromosome that is joined to another chromosome. Example: Burkitt's lymphoma, a cancer of human B-lymphocytes.
- **Overexpression of growth factor receptors:** Some oncogenes encoding growth factor receptors have been identified. Overexpression and structural alterations in growth factor receptors are associated with carcinogenesis. In lung cancer, gene erb-B (which encodes for ECG-receptor) overexpression is observed.

  The mutation of ras proto-oncogene is the most important cause of many human tumors. The inactive ras protein is in a bound state with GDP (guanidine diphosphate). When the cells are stimulated by growth factors, ras P21 gets activated by exchanging GTP (catalyzed by GRF-guanine nucleotide releasing factors). The active ras P21 stimulates regulators such as cytoplasmic kinases. This leads to DNA replication and cell division.

  The activity of ras P21 in normal cells is short-lived. Hydrolysis of GTP to GDP by GTPase activity reverts ras P21 to the normal origin state. Point mutations in the ras gene result in the production of altered ras P21 (lacking GTPase activity). This leads to permanent activation of ras P21, leading to uncontrolled multiplication of cells.

- **Gene amplification:** Several amplifications of certain DNA sequences are observed in some cases of cancer.
- **Point mutation:** Change in a single base in DNA leads to point mutation. The ras proto-oncogene is an example of activation by point mutation. The mutated ras oncogene produces a protein (GTPase) that differs in structure by a single amino acid. This alteration diminishes the activity of GTPase, a key enzyme involved in the control of cell growth.

CHARACTERISTICS OF GROWING TUMOR CELLS

BAQ: Describe the characteristics of growing tumor cells.

Ans: Following are the general and morphological changes observed in cancer:

1. **Shape of cells:** The tumor cells are much rounder in shape (compared to normal cells).
2. **Loss of contact inhibition:** The normal cells are characterized by contact inhibition (they form monolayers). The cancer cells form multi-layers due to loss of contact inhibition. Cancer cells can move freely and get deposited in any part of the body (metastasis).
3. **Loss of anchorage dependence:** The cancer cells can grow without attachment to the surface, while normal cells firmly adhere to the surface.
4. **Alteration in permeability properties:** The tumor cells have altered permeability and transport across the membrane.

The following are the main biochemical changes observed in cancer:

1. **Increased replication and transcription:** The synthesis of RNA and DNA is increased in cancer cells (i.e. an increase in anabolic processes).
2. **Increased glycolysis:** There are increased energy demands of multiplying cells and it is indicated by an elevation in aerobic and anaerobic glycolysis.
3. **Enzyme alterations:** The activities of certain enzymes are changed, e.g. proteases.
4. **Decreased pyrimidine metabolism:** A reduction in certain catabolic reactions is observed (e.g., reduction in degradation of pyrimidines in tumor cells).
5. **Alteration in antigens:** A loss of regularly occurring antigens coupled with the appearance of new antigens is observed in tumor cells.
6. **Alterations in the structure of molecules:** Changes are observed in the structure of glycoproteins of disease status after the initial therapy and in the monitoring of subsequent treatment modalities.

MULTI-STEP PROCESS OF CANCER

BAQ: Write on the system that describes the various stages of cancer.

Ans: The stage of a cancer is described usually as numbers I to IV, depending on how much the cancer has spread. The stage often takes into account

1. The size of a tumor
2. How deeply the tumor has penetrated
3. Whether it has invaded adjacent organs
4. How many lymph nodes it has metastasized, and
5. Whether it has spread to distant organs.

The TNM classification describes the cancer. ‘T’ describes the size of a tumor and whether it has invaded nearby tissue. ‘N’ describes the involved regional lymph node, and ‘M’ describes metastasis.

Staging of cancer is important because the stage at diagnosis is the most powerful predictor of survival, and treatments are often changed based on the stage. Invasive cancers that are confined within the wall of the colon (TNM stages I and II) are curable with surgery. If untreated, they spread to regional lymph nodes (stage III), where up to 73% are curable by surgery and chemotherapy. Cancer that metastasizes to distant sites (stage IV) is usually not curable. However, chemotherapy and radiotherapy can extend survival, and in rare cases, surgery and chemotherapy together are effective.

CANCER TREATMENT

LAQ: Write a note on cancer treatment and related symptoms.

Ans: Various types of treatments used to treat cancer are chemotherapy, radiotherapy, and immunotherapy.

Chemotherapy

Chemotherapy is the treatment of cancer by chemicals. Most commonly, chemotherapy acts by killing cells that divide rapidly. Chemotherapy also harms cells that divide rapidly under normal circumstances, i.e. cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side effects of chemotherapy:

- Mucositis (inflammation of the lining of the digestive tract)
- Myelosuppression (decreased production of blood cells)
- Alopecia (hair loss)

Examples of chemotherapy drugs: Alkylating agents such as altretamine, bendamustine, busulfan, carboplatin, etc. damage the DNA of a cancer cell to prevent cancer cells from dividing.

Antimetabolite drugs such as Azacitidine, capecitabine, cladribine, etc. prevent cancer cells from synthesizing the genetic material cells require to divide.

The main ways used for chemotherapy are:

1. Oral treatment is given in the form of pills, capsules, or liquids.
2. Intravenous (IV) treatment by introducing the drug into a vein.
3. By injecting a drug in a muscle of the arm, thigh, or hip, or under the skin in the fatty part of the arm, leg, or belly.

Chemotherapy is useful to:

1. Reduce the size of a tumor before surgery or radiation therapy.
2. Destroy cancer cells that may remain after radiation therapy or surgery
3. Destroy cancer cells that appear again or spread to different parts of the body to support other types of treatments.

The following are common side effects of chemotherapy:

1. **Depression of the immune system:** This can result in potentially fatal infections.
2. **Fatigue:** The treatment can be physically exhausting for the patient.
3. **Anemia:** Chemotherapy may produce mild to severe anemia.
4. **Tendency to bleed easily:** Medications that kill rapidly dividing cells or blood cells are likely to reduce the number of platelets in the blood, which can result in bruises and bleeding.
5. **Gastrointestinal distress:** Nausea and vomiting are common side effects of chemotherapeutic medications that kill fast-dividing cells. This can also produce diarrhea or constipation.
6. **Malnutrition and dehydration:** The patient may suffer from malnutrition when the patient does not eat or drink enough due to nausea or may suffer from dehydration when the patient vomits frequently, because of gastrointestinal damage.
7. **Hair loss:** Some medications that kill rapidly dividing cells cause dramatic hair loss. This effect is temporary. Hair usually starts growing back a few weeks after the last treatment.
8. The drugs used for chemotherapy can be hepatotoxic, cardiotoxic, nephrotoxic, or ototoxic.

Radiation therapy

Radiation has been used for several years to attack cancer cells where they live. It uses high-energy X-rays, radioactive isotopes, and electron beams. The genetic makeup of cancer cells is destroyed or damaged at the chromosome level by radiation therapy.

Radiotherapy is called a local treatment. That means it occurs where the disease is found. Radiation is directed at the cancer cells.

Radiation treatment and chemotherapy have both proven to be more effective when they are used together. Radiation enhances the effects of chemotherapy. A common use is directed at shrinking cancerous growths before they are removed surgically. For tumors that cannot be removed from the body, radiation can still be used to reduce pain, blockages, and bleeding caused by cancerous tumors.

Side effects of radiotherapy: Radiation therapy side effects tend to be localized. Their greatest effects are felt in the areas of the body where the therapy is applied. Effects can include localized irritation to areas like the skin, hair, neck, or chest or vomiting, nausea, and diarrhea. Medicinal and other techniques have been developed to minimize many of the side effects that radiation can cause.

IMMUNOTHERAPY

In immunotherapy for cancer treatment, substances such as monoclonal antibodies synthesized by a patient's body cells or prepared in a laboratory are used to boost the immune system and help the immune system of the patient to find and destroy cancer cells. Various types of drugs used in immunotherapy are:

1. Immune checkpoint inhibitors, that block immune checkpoints. By this means these drugs allow immune cells to respond more strongly to cancer.
2. T-cell transfer therapy that boosts the natural ability of T cells to fight cancer.
3. Monoclonal antibodies, which are immune system proteins created in the laboratory that are designed to bind to specific targets on cancer cells.
4. Use of vaccines, which work against cancer by boosting the immune system's response to cancer cells.
5. Immune system modulators, which increase the immune response of the patient's body against cancer.

The following are the side effects of immunotherapy

- Nausea
- Vomiting
- Headache
- Fever
- Muscle pain
- Joint pain
- Weakness
- Fatigue, etc.

TUMOR MARKERS

Competency achievement: The student should be able to:

BI10.2: Describe various biochemical tumor markers and the biochemical basis of cancer therapy

LAQ: Describe various biochemical tumor markers and the biochemical basis of cancer therapy.

Ans: A tumor marker is a biochemical substance produced by a tumor, which can be detected in body fluids such as blood, urine, cerebrospinal fluid (CSF), and cells and body tissues. A tumor marker may be made by a tumor itself, or it may be made by the body as a response to the tumor.

Cancer that is detected early can potentially be cured. The goal of the treatment is to diagnose cancer when a tumor is small enough to be completely removed surgically. Unfortunately, most cancers do not produce symptoms until the tumors are either too large to be removed surgically or until cancerous cells have already spread to other tissues (metastasized). An ideal tumor marker should be both specific for a given type of cancer and sensitive enough to detect small tumors for early diagnosis or during screening.

Tumor markers can be measured qualitatively or quantitatively by immunological, chemical, or molecular biological methods to determine the presence of cancer. Markers produced by cancers include enzymes and isoenzymes, hormones, oncofetal antigens, carbohydrate epitopes, etc.

Clinical applications of tumor markers are as follows:

Tumor markers are useful in:

1. Clinical staging of cancer
2. Differential diagnosis in symptomatic patients.
3. Screening in the general population.
4. Estimating tumor volume.
5. Prognostic indicator for disease progression.
6. Evaluating the success of the treatment
7. Detecting the recurrence of cancer
8. Monitoring responses to therapy
9. Determining the direction for immunotherapy.

Enzymes as tumor markers: Enzymes are present in much higher concentrations inside than outside the cell. Enzymes are released into the cell's systemic circulation as the result of tumor necrosis or due to the change in the permeability of cancer cells. Increased enzyme levels are also observed in the blockage of pancreatic or biliary ducts and also in renal insufficiency. Most enzymes, however, are not unique to a specific organ. Isoenzymes and multiple forms of enzymes may provide additional organ specificity. Determination of the following enzymes can be helpful in the evaluation of specific cancers.

- **Alkaline phosphatase (ALP):** Elevated levels of ALP are seen in primary or secondary liver cancer. Its level may help evaluate metastatic cancer with bone or liver involvement.
- **Creatine kinase (CK):** CK1, an isoenzyme of CK, is present in the brain, prostate gland, gastrointestinal tract, lung, bladder, uterus, and placenta. Elevated values of CK1 have been observed in the prostate and small cell carcinoma of the lung. It also may be elevated in malignancies of the breast, colon, ovary, and stomach.
- **Lactate dehydrogenase (LDH):** Elevated values of serum LDH are observed in cancer of the liver, acute leukemia, breast, colon, stomach, and lung. The serum LDH levels have been shown to correlate with tumor mass in solid tumors and provide a prognostic indicator for disease progression.
- **Prostatic acid phosphatase (PAP):** Elevated values of serum PAP are seen in prostatic cancer. In other malignant conditions such as multiple myeloma, bone metastases of other cancers, and osteogenic sarcoma also, high values of serum PAP are observed.

Hormones as tumor markers: The production of hormones in cancer involves two separate routes. It is produced by the tissue of the organ, which produces it normally, and it may be produced by nonendocrine tissue. This latter condition is called ectopic syndrome. For example, ACTH is produced by the pituitary gland and also by a small cell of the lung. Thus, elevated serum levels of ACTH could be the result of pituitary or ectopic production.

Table 16.2: Hormones as tumor markers

Hormones	Type of cancer
ACTH	• Cushing's syndrome, lung (small cell)
ADH	• Procaryotic, duodenal, adrenal cortex, lung (small cell)
Calcitonin	• Medullary thyroid
Gastrin	• Glucagonoma
hCG	• Embryonal, testicular choriocarcinoma
Parathyroid hormones	• Liver, renal, breast, lung, etc.
Prolactin	• Renal, lung, pituitary adenoma
Growth hormone	• Renal, lung, pituitary adenoma
Human placental	• Gonads, lung, breast, lactogen trophoblastic

Oncofetal antigens: Oncofetal antigens are proteins produced during fetal life. These proteins are present in high concentrations in the sera of fetuses and decrease to low levels or decrease after birth. Malignant transformation of cells results in the reactivation of certain genes, which synthesize oncofetal antigens. Following are some of the important oncofetal antigens:

- **α-fetoprotein (AFP):** It is a marker for hepatocellular and germ-cell carcinoma Elevated levels of AFP < 200 µg/L are also associated with hepatitis and cirrhosis of the liver. AFP levels> 1000 mmg/L are indicative of cancer. The levels of AFP correlate with the size of the tumor, hence its determination helps to detect the early stages of the cancer. AFP is also useful for determining prognosis and in the monitoring of therapy for hepatocellular carcinoma.
- **Carcinoembryonic antigen:** CEA is a marker for colorectal, gastrointestinal, lung, and breast carcinoma. CEA testing may be useful as an adjunct to clinical staging. Persistently elevated levels that are 5 to 10 times the upper reference limit strongly suggest the presence of colon cancer. CEA is also useful in monitoring breast, lung, gastric and pancreatic carcinoma.

Carbohydrates as tumor markers: These markers represent a new generation of clinically useful tumor markers. They tend to be more specific than naturally secreted markers such as enzymes and hormones. These are either antigens on the tumor cell surface or those that are secreted by the tumor cells.

- CA 15-3 is a marker for breast carcinoma. It should not be used to diagnose primary breast cancer, since the incidence of elevation is fairly low. CA 15-3 is most useful in monitoring therapy and disease progression in metastatic breast cancer patients. Elevated levels of CA 15-3 are also found in other malignancies, including pancreatic, lung, ovarian, colorectal, and liver cancer.
- CA 125 is a marker for ovarian and endometrial carcinomas. It is also elevated in pancreatic, lung, breast, colorectal, and other gastrointestinal tumors. In the detection of recurrent metastasis use of CA 125 as an indicator is about 75% accurate. CA 125 correlated with disease progression or regression in 80 to 90% of cases.

BAQ: Enumerate four blood group antigens that can be used as tumor markers.

Ans: The following are blood group antigens used as tumor markers:

1. CA 19–9 is a marker mainly for both colorectal and pancreatic carcinoma. Elevated levels are also seen in patients with hepatocellular, hepatobiliary, gastric, and breast cancer.
2. CA 50 is a marker for pancreatic and colorectal carcinoma. Elevated levels of CA 50 have been reported in benign diseased cases of the pancreas, biliary tract, and liver.
3. CA 72-4 is a marker for carcinomas of the gastrointestinal tract and of the ovary.
4. CA 242 is a marker for pancreatic and colorectal cancer. Significant increases are also observed in patients with benign colic, gastric, hepatic, pancreatic, and biliary tract diseases.

SAQ: What are Bences Jones proteins? What is the significance of testing urine for Bence Jones proteins?

Ans: Bence Jones proteins are light chain immunoglobulin (paraproteins) and are produced by neoplastic plasma cells. They can be kappa (most of the time) or lambda. The light chains can be immunoglobulin fragments or single homogeneous immunoglobulins. They are found in urine due to decreased filtration capabilities of kidneys, often induced by hypercalcemia from the calcium released as the bones are destroyed. Detection of Bence Jones proteins in urine indicates multiple myeloma.

SAQ: What is the significance of the determination of serum prostate-specific antigen (PSA)?

Ans: Increased serum PSA values indicate prostate cancer. Prostate cancer is the leading cancer in older men. Early detection is important, and when detected early, prostate cancer is potentially curable by radical prostatectomy. PSA is found in prostatic tissue and it is organ-specific (not present in other tissues). PSA is a single-chain glycoprotein.

Multiple Choice Questions

Q1. Which of this cancer does not form a solid neoplasm?

A. Lymphoma B. Leukemia
C. Sarcoma D. Lipoma

Q2. Cancer is often the result of the activation of

A. Proto-oncogenes
B. Oncogenes
C. Tumor-suppressor genes
D. All of the above

Q3. About 50% of all human cancers may involve an abnormal or missing

A. Oncogene
B. Proto-oncogene
C. p53 gene
D. BRCA-1 gene

Q4. Cancer may be the result of inactivation of

A. Proto-oncogenes
B. Oncogenes
C. Tumor-suppressor genes
D. All of the above

Q5. Telomerase is active in the cells of

A. Blood B. Sperm
C. Muscle D. Bone

Q6. All of the following are characteristic of cancer cells *except*

A. Loss of cell cycle control
B. Transplantability
C. Loss of contact inhibition
D. Contact inhibition

Q7. The Philadelphia chromosome is associated with which type of cancer?

A. Breast B. Leukemia
C. Prostate D. Thyroid

Q8. BRCA-1 is associated with cancer of

A. Breast B. Thyroid
C. Prostate D. Blood

Q9. The p53 protein normally promotes

A. DNA repair B. Tumor formation
C. Cell division D. Apoptosis

Q10. The p53 gene is especially prone to

A. Inactivation
B. Chromosomal rearrangement
C. Point mutation
D. A and C

Q11. Which of the following may contribute to causing cancer?

A. Unrestrained control of growth of cells
B. Faulty DNA repair
C. Inactivation of p53
D. All of the above

Q12. Elevated levels of serum alkaline phosphatase are seen in

A. Liver cancer
B. Prostate cancer
C. Bladder cancer
D. B and C

Q13. Elevated levels of which serum enzyme are observed in prostate cancer?

A. Alkaline phosphatase
B. Amylase
C. LDH
D. Acid phosphatase

Q14. Elevated levels of which serum enzyme are observed in pancreatic cancer?

A. Alkaline phosphatase
B. Amylase
C. LDH
D. Acid phosphatase

Q15. High levels of serum ACTH could be the result of

A. Ectopic production
B. Pituitary tumor
C. Prostate cancer
D. A or B

Q16. α-fetoprotein is a marker of

A. Pancreatic cancer
B. Hepatocellular carcinoma
C. Germ cell carcinoma
D. B and C

Q17. Determination of progesterone and estrogen receptors is useful in

A. Breast cancer B. Prostate cancer
C. Bladder cancer D. Liver cancer

Q18. Exposure of DNA to ultraviolet radiation can lead to the formation of

A. Adenine dimers B. Thymine dimers
C. Guanine dimers D. Uracil dimers

Q19. Damage to DNA caused by ultraviolet radiation can be repaired by

A. DNA polymerase
B. UvrABC excinuclease
C. DNA ligase
D. All of the above

Q20. Substitution of an adenine base by guanine in DNA is known as

A. Transition B. Transposition
C. Transversion
D. Frameshift mutation

Q21. Substitution of a thymine base by adenine in DNA is known as

A. Transposition B. Transversion
C. Transition
D. Frameshift mutation

Q22. A point mutation results from

A. Deletion of a base
B. Insertion of a base
C. Substitution of a single base nucleotide
D. All of these

Q23. Substitution of a base can result in

A. Nonsense mutation
B. Mis-sense mutation
C. Silent mutation D. All of these

Q24. The effect of a mis-sense mutation can be

A. Unacceptable
B. Partially acceptable
C. Acceptable D. A, B, or C

Q25. Amino acid sequence of the encoded protein is not changed in

A. Mis-sense mutation
B. Silent mutation
C. Both A and B
D. None of these

Q26. Mutations in promoter regions of genes can cause

A. Decreased efficiency of transcription
B. Change in the reading frame of a downstream structural gene
C. Premature termination of translation
D. All of these

Answers

1. B **2.** A **3.** C **4.** C **5.** B **6.** D **7.** B **8.** A **9.** D **10.** D
11. D **12.** A **13.** D **14.** B **15.** D **16.** D **17.** A **18.** B **19.** D **20.** A
21. B **22.** C **23.** D **24.** D **25.** B **26.** A

Chapter

17

Organ Function Tests

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

Organ function tests such as kidney function tests, liver function tests, etc. are useful for the following purposes:

1. In the differential diagnosis of the different types of diseases related to a specific organ.
2. To assess the severity of organ damage.
3. To follow the trend of the disease.
4. To gauge post–operative risk.
5. To diagnose the presence of latent disease, related to a specific organ.
6. To screen the suspected cases during the outbreak of specific viral diseases.
7. To screen patients exposed to potentially toxic materials in the industry.
8. To screen the patients exposed to drugs used to treat any specific disease.

RENAL FUNCTION TESTS

Competency achievement: The student should be able to:

PY7.8: Describe and discuss renal function tests

Kidney Functions

LAQ: Describe and discuss renal function tests.

Ans: Assessment of the overall function of the kidneys is based on the assumption that all functioning nephrons are performing normally and that decline in renal function is due to the loss of functioning nephrons quantitatively related to the loss. In most of the types of diffuse renal disease, impaired function of kidneys is due to a diminished number of functioning nephrons. The various kidney functions are deranged in prerenal, renal, and post-renal conditions. It may not be possible to find out the exact reason for the deranged functions of kidneys based on biochemical tests, but it may be possible to find out which of the following functions of kidneys are disturbed:

1. Formation of normal urine
2. Excretion of the normal amount of urine
3. Glomerular filtration
4. Acidification of the urine
5. Selective absorption
6. Secretion of certain substances
7. Homeostatic states of the body
8. Normal endocrine functions

In an organ as complex as a kidney, there may be many processes that may be impaired, and it may be necessary for a physician to know the location of the defects. Clinical signs and symptoms may be minimum or absent or, if present, may not always reflect the severity of the kidney disease or the prognosis for the patient. The diagnosis of kidney disease, to a great extent, is made in the clinical chemistry laboratory. The kidney function tests, when properly conducted, can give valuable information about the status of kidney function and frequently about the location of the defect. However, the kidney has a considerable functional reserve, and these tests may be normal even in the presence of relatively severe renal pathology.

Kidney function tests, in general, can be affected by prerenal, renal, or post-renal causes. In prerenal conditions, due to decreased plasma volume (dehydration) or due to decreased blood flow (excessive bleeding, shock, cardiac failure, etc.), decrease in kidney function is observed.

Among the renal causes for decreased kidney function are:

1. The conditions affecting glomerular filtration rate or
2. The tubular function or
3. Any changes in the renal vascular system that decreases the blood flow.

A post-renal cause for the decrease in kidney function is the obstruction of the urine flow; it may be caused by enlargement of the prostate, stones in the urinary tract, or tumors of the bladder. In these cases, the decreased kidney function is due to a reduction in the effective filtration pressure of the glomeruli. It is possible to distinguish between renal insufficiency and renal failure. Renal insufficiency may be considered to be present when the plasma levels of excreted end products are still normal, while in renal failure (usually when clearance has fallen below 50%), these plasma components, e.g. urea, are above normal plasma levels.

Kidney function tests: Following are the routinely performed kidney profile tests:

1. Blood urea nitrogen
2. Serum creatinine
3. Routine urine examination
4. Serum total proteins, albumin, globulin, A/G ratio
5. Creatinine clearance test

Clinical Significance of Tests

1. Blood urea nitrogen
2. Serum creatinine and
3. Routine urine examination

These tests can help differentiate prerenal conditions from renal and post-renal conditions.

The normal ratio of urea nitrogen and serum creatinine is observed to be 14 to 24.

a. In cases of retention of urea nitrogen due to prerenal causes, the ratio is generally observed to be higher and maybe up to 40.
b. In the absence of prerenal conditions, however, during the early stages of renal disease, the urea nitrogen/creatinine ratio may be normal since the tubules can secrete raised serum creatinine to a level. In the presence of normal renal blood flow, any increase in creatinine values above 2 mg/dl is suggestive of moderate to severe kidney damage, and in severe tubular damage, the ratio may be as low as 10.
c. Retention of urea and creatinine due to obstruction of the urinary tract in post-renal conditions will cause a simultaneous and proportional increase in both serum urea nitrogen and creatinine levels. The ratio will be below 14, depending upon the percentage of obstruction to urea excretion.
d. Examination of urine for protein, cells, and casts gives an idea of the presence of an 'active lesion' in the kidneys.
e. Once the renal damage has been detected, renal function tests may reveal the principal site and degree of the disturbance in the nephron.

Test no. 4: Serum total proteins, albumin, globulin, A/G ratio

Clinical significance: In nephritis type I (Glomerulonephritis), moderate loss of proteinuria is observed, and in nephritis type II (nephrotic syndrome) massive loss of proteinuria is observed. A typical electrophoretic protein pattern with decreased albumin and gamma globulin but increased beta and alpha-2 globulin indicates nephritis.

Test No. 5: Creatinine clearance test

Creatinine clearance test gives a measure of the actual excretory capacity of the kidney; since this test measures the amount of a substance excreted in the urine as compared to the concentration of the same substance in the plasma. Normal values for creatinine clearance are 105 ± 20 ml/minute for males and 95 ± 20 ml/minute for females.

Creatinine clearance values less than 50 ml are of graver prognostic significance. Creatinine clearance values less than 50% of the normal average indicate significant renal dysfunction and creatinine clearance falls below 20% in severe renal failure.

Multiple Choice Questions

Q1. Increase in all these substances in blood indicates "renal condition", *except*

A. Urea
B. Glucose
C. Creatinine
D. Uric acid

Q2. Water reabsorption in distal tubules is regulated by

A. ADH
B. FSH
C. Parathyroid hormones
D. A, B and C

Q3. These hormones increase the reabsorption of calcium ions and decrease the reabsorption of phosphate ions at kidney level

A. Mineralocorticoids
B. Parathyroid hormones
C. Corticosteroids
D. Androgens

Q4. pH of freshly voided urine (sample collected after 8–12 hours of fasting) is

A. Acidic
B. Basic
C. Neutral
D. B or C

Q5. Normal ratio of serum urea nitrogen/serum creatinine is

A. > 40
B. 14–24
C. 5–10
D. > 5

Q6. When the ratio of serum urea nitrogen/serum creatinine is >40, it may be due to

A. Renal condition
B. Prerenal condition
C. Postrenal condition
D. A or C

Q7. Which of the following proteins increase in blood in nephritis?

A. Albumin
B. Beta-globulin
C. alpha-2 globulin
D. B and C

Q8. Normal average glomerulus filtration rate (GFR) is

A. 80
B. 125
C. 210
D. 50

Q9. Osmolarity is a measure of the concentration of which of these components in serum?

A. Total concentration of dissolved particles
B. Protein-bound particles
C. Only sodium and chloride ions
D. Only calcium and phosphate ions

Q10. Which of the following tests gives an idea about the excretory capacity of kidneys?

A. Serum total protein
B. Serum albumin
C. Creatinine clearance
D. Serum creatinine

Answers

1.B **2.** A **3.** B **4.** A **5.** B **6.** B **7.** D **8.** B **9.** A **10.** C

Case Studies

Case 1

A 36-year-old man was admitted to hospital after a building collapse incident. He suffered from severe body injuries and on examination, it was found that he was dehydrated and in a state of shock. He had a swollen and tender abdomen. His laboratory test reports were as follows:

	Reference range (Normal values)
Serum urea nitrogen (SUN): 63 mg/dl	7–21 mg/dl
Serum creatinine: 1.4 mg/dl	0.6–1.2 mg/dl
Serum total proteins: 5.0 g/dl	6–8 g/dl
Serum albumin: 2.4 g/dl	3.3–4.8 g/dl
Serum total globulins: 2.6 g/dl	1.8–3.6 g/dl

Glomerular filtration rate (GFR): 50 ml

1. What is the probable diagnosis?

Ans: Pre-renal condition, since serum urea nitrogen/serum creatinine = 45. When this ratio is >40, it indicates **pre-renal condition**, due to significant loss of blood in the accident.

2. What is the mechanism behind elevated values of SUN and S. Creatinine? And a decrease in GFR?

Ans: Internal bleeding has led to a significant decrease in blood circulation to the kidneys, and a decrease in GFR. Due to a decrease in GFR, SUN, and S. creatinine have increased.

3. What is the first line of treatment?

Ans: Fluid replacement by IV saline and appropriate blood transfusion.

BAQ: Show horizontal integration of symptoms and test reports of Case 1 with anatomy and physiology.

Ans:

Horizontal integration with anatomy

Affected kidneys, due to insufficient blood circulation

Horizontal integration with physiology

Decrease in glomerular filtration rate (GFR)

BAQ: Show vertical integration of symptoms and test reports of this patient with general medicine, pathology, and transfusion medicine.

Ans:

Vertical integration with general medicine

Study and management of pre-renal conditions

Vertical integration with pathology

Diagnosis and prognosis of case 1

Vertical integration with transfusion medicine: Testing of blood group, rho (D), and appropriate blood transfusion.

Case 2: A 36-year-old woman was admitted to the hospital with significant weight loss, lethargy, and generalized weakness. On examination, the patient was found to be anemic and had a blood pressure of 175/110 mmHg. Random urine examination showed the presence of proteins (++) and the absence of glucose. Her laboratory test reports were as follows:

	Reference range Normal values
Serum urea nitrogen: 110 mg/dl	7–21 mg/dl
Serum creatinine: 9.7 mg/dl	0.6–1.2 mg/dl
Serum total proteins: 5.0 g/dl	6–8 g/dl
Serum albumin: 2.4 g/dl	3.3–4.8 g/dl
Serum total globulins: 2.6 g/dl	1.8–3.6 g/dl

Glomerular filtration rate (GFR): 80 ml

1. What is the probable diagnosis?

Ans: Very high values of blood urea nitrogen, serum creatinine, decreased serum proteins, low GFR, and the presence of a significant amount of protein in the urine indicate that the patient is suffering from **severe renal disease.**

2. What is the biochemical basis for the increase in serum urea nitrogen and serum creatinine?

Ans: The increase in serum urea nitrogen and creatinine is due to the decrease in glomerular filtration rate (GFR), due to severe renal disease.

3. Why blood pressure (BP) of this patient increased?

Ans: Due to renin secretion increases the flow of blood to kidneys (action of renin-angiotensin system mechanism), leading to an increase in BP.

4. What is the mechanism behind the loss of protein in urine?

Ans: Blood proteins are leaked through the damaged basement membrane of glomeruli in severe renal disease.

5. What is the probable line of treatment?

Ans: It is necessary to find out the cause of "renal disease" and then according to the cause of the disease is treated.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy, physiology, and biochemistry.

Ans:

Horizontal integration with anatomy

Kidney disease, glomerular damage (since serum proteins were decreased)

Horizontal integration with physiology

Oligourea, and proteinurea

Horizontal integration with biochemistry

Increase in renin secretion and related action on the angiotensin system.

Horizontal integration with nutrition

A suggestion of a diet low in salt and a controlled intake of proteins

BAQ: Show vertical integration of symptoms and test reports of this patient with general medicine and pathology.

Ans:

Vertical integration with general medicine

Study and management of renal disease

Vertical integration with general medicine

Diagnosis and prognosis of Case 2

Case 3: A 68-year-old man was presented with a complaint of difficulty in passing urine. Ultrasound report indicated the presence of large calculi in both ureters. His laboratory test reports were as follows:

	Reference range Normal values
Serum urea nitrogen (SUN): 17 mg/dl	7–21 mg/dl
Serum creatinine: 0.9 mg/dl	0.6–1.2 mg/dl
Fasting urine volume: 20 ml	60–200 ml
Urine proteins: Absent	Absent

1. What is the probable diagnosis?

Ans: Report of ultrasound, low amount of excretion of urine (oliguria) and normal reports of serum urea nitrogen and serum creatinine indicate **post-renal condition.**

2. Why levels of serum urea nitrogen and serum creatinine are normal in this case?

Ans: At this stage, kidney functions are normal. Hence, SUN and S. creatinine are normal.

3. What is the mechanism due to which the patient suffered from oliguria?

Ans: Due to compression of ureters by the urinary calculi, the quantity of urine excretion was reduced.

4. What is the probable line of treatment?

Ans: Removal of the calculi in the ureters.

SAQ: Show horizontal integration of symptoms and test reports of Case 3 with physiology.

Ans:

Horizontal integration with physiology

Oliguria. Decrease in the excretion of urine.

BAQ: Show vertical integration of symptoms and test reports of Case 3 with general medicine and pathology.

Ans:

Vertical integration with general medicine

Study and management of post-renal conditions.

Vertical integration with pathology

Study of cause, diagnosis, and prognosis of Case 3.

Case 4: A 20-year-old girl was admitted to the hospital with edema on her face and feet, lethargy, headache, and generalized weakness. Her blood pressure was 170/110.

On examination of the case history, it was found out that she was using fairness cream provided by a private beauty parlor. Random urine examination showed the presence of proteins (+++). Her other laboratory test reports were as follows:

	Reference range (Normal values)
Serum urea nitrogen: 90 mg/dl	7–21 mg/dl
Serum creatinine: 6.8 mg/dl	0.6–1.2 mg/dl
Serum total proteins: 4.8 g/dl	6–8 g/dl
Serum albumin: 2.3 g/dl	3.3–4.8 g/dl
Serum total globulins: 2.5 g/dl	1.8–3.6 g/dl

Glomerular filtration rate (GFR): 70 ml

1. What is the probable diagnosis?

Ans: Very high values of blood urea nitrogen and serum creatinine, decreased GFR, very low serum proteins, and the presence of a significant amount of protein in the urine indicates that the patient is suffering from **severe renal disease.**

2. Why blood pressure (BP) of this patient was increased?

Ans: GFR is very low. BP was increased due to renin secretion to increase the flow of blood to kidneys (action of renin-angiotensin system mechanism).

Q3. What is the biochemical basis for renal disease?

Ans: Due to the regular use of skin-fair cream, the level of her blood mercury must be more than the toxic level (> 150 µg/L). A very high percentage of mercury in blood was probably responsible to damage kidneys and related functions (GFR: 70). Acceptable range of mercury in blood is < 20 µg/L. Many private beauty parlors use fairness creams, that are not approved by FDA. These fairness creams contain a very high percentage of mercury. Melanin is produced by the melanocytes in the skin in a reaction catalyzed by the enzyme tyrosinase during the conversion of tyrosine into dopa. Melanin gives dark color to the skin. Mercury in skin-fair cream denatures tyrosinase and melanin formation decreases, leading to fair skin.

Q4. What is the first line of treatment?

Ans: It is necessary to stop the use of skin-fair cream immediately and then provision of supportive therapy for kidney, respiratory and cardiovascular functions.

BAQ: Show horizontal integration of symptoms and test reports of Case 4 with anatomy, physiology, and biochemistry.

Ans: Horizontal integration with anatomy

Kidney disease, glomerular damage (since serum proteins were decreased)

Horizontal integration with physiology

Oligourea, and proteinurea

Horizontal integration with biochemistry

Ans: Increase in blood mercury above toxic level. Increase in renin secretion and related action on the angiotensin system.

Horizontal integration with nutrition

The suggestion of diet low in salt and controlled intake of proteins.

BAQ: Show vertical integration of symptoms and test reports of Case 4 with general medicine and pathology.

Ans:

Vertical integration with general medicine

Study and management of renal disease caused by toxic chemicals

Vertical integration with pathology

Study of cause, progress, and prognosis of renal disease caused by toxic chemicals.

Case 5: A 29-year-old man, a bodybuilder, presented with edema on his face and feet, nausea, vomiting, fatigue, and shortness of breath. His blood pressure was 165–95. From his case study, it was found that he was on high-protein supplements containing branch-chain amino acids. His laboratory test values were as follows:

	Reference range (Normal range)
Serum urea nitrogen: 110 mg/dl	7.0–21 mg/dl
Serum creatinine: 8.7 mg/dl	0.7–1.5 mg/dl
Urine proteins: Present +++	Absent

1. What is the probable diagnosis?

Ans: Very high values of blood urea nitrogen and serum creatinine, indicate that the patient is suffering from **severe renal disease.**

2. What is the biochemical basis for renal disease?

Ans: Long-term inappropriate high dietary protein intake can cause intraglomerular hypertension, which may result in kidney hyperfiltration, glomerular injury, and proteinuria. Long-term high protein intake may lead to chronic kidney disease.

Branched-chain amino acids (BCAA) are used by athletes to improve athletic performance, prevent fatigue, improve concentration, and reduce muscle breakdown during intense exercise. But there is limited scientific research to support the benefits of BCAA. High protein supplements with BCAA should be used cautiously under appropriate medical supervision.

3. What is the first line of treatment?

Ans: It is necessary to stop the high intake of protein supplements immediately and then the provision of supportive therapy for kidney, respiratory and cardiovascular functions.

Competency achievement: The student should be able to:

PY4.8: Describe and discuss gastric function tests, pancreatic exocrine function tests, and liver function tests

LIVER FUNCTION TESTS

Liver Functions

BAQ: Describe liver functions.

Ans: Functions performed by the liver are:

1. **Excretory functions:**
   - A. Bile formation and excretion of bile into the intestine.
   - B. Secretion of products in the bile emanating from the liver parenchymal cells (which comprises 60% of its mass), e.g. bile salts, bilirubin conjugates, and cholesterol.
   - C. Excretion of substances withdrawn from the blood by hepatic activity, e.g. heavy metals, dyes such as bromsulfthalein, and alkaline phosphatase.
2. **Metabolic functions:** The liver is the center of metabolic activity for carbohydrates, protein, and lipids.
   - A. *Carbohydrates:* Sugars and carbon residues from protein and fat are converted to glycogen. Glycogen is stored as a carbohydrate reserve.
   - B. *Proteins:* Amino acids are deaminated, and ammonia is converted to urea. Immunoglobulins are synthesized in the cells of the reticuloendothelial system. Albumin is synthesized by the parenchymal cells.
   - C. *Lipids:* The liver contains a store of neutral fat. Synthesis of cholesterol, bile acids, fats, and lipoproteins take place in the liver. The liver also plays an important role in the esterification of cholesterol and other lipids.
3. **Protective functions and detoxication**
   - A. Kupffer cell activity in removing foreign bodies from blood (phagocytosis).
   - B. Detoxication by conjugation, methylation, oxidation, and reduction.
   - C. Removal of ammonia from blood, particularly that absorbed from the intestine by way of the portal vein. Ammonia formed in the deamination of amino acids is converted to urea, which is excreted through urine.
4. **Hematologic functions:** (Hematopoiesis and coagulation)
   - A. Blood formation in the embryo (and in some abnormal states in the adults.)
   - B. Production of blood coagulation factors

   (I to XIII, PK, HMWK, etc.), fibrinogen, prothrombin, and heparin, which play an important role in blood coagulation.
   C. Destruction of erythrocytes (at the end of their respective lifespan).
5. **Circulatory functions**
   A. Transfer of blood from the portal to the systemic circulation.
   B. Activity of its reticuloendothelial system (Kupffer cells) in immune mechanisms.
   C. Blood storage (regulation of blood volume).

Q: Write a note on the metabolism of bile acids.

Ans: The following are the various steps involved in the metabolism of bile acids:

1. Approximately one-third of the daily production of cholesterol is converted into bile acids. The bile acids synthesis rate averages 200 to 400 mg/day.
2. The first step in bile acid synthesis involves the rate-limiting step, 7-alpha-hydroxylation, and 7-hydroxycholesterol formation.
3. 7-hydroxycholesterol is converted to the primary bile acids–cholic acid and chenodeoxycholic acid. They get conjugated with glycine or taurine and form glycocholic acid and taurocholic acid, respectively, and enter the bile canaliculi, and then these are present in bile and stored in the gall bladder.
4. In the bile, these bile acids exist as bile salts (sodium or potassium salts of conjugated bile acids since the pH of bile is alkaline). The bile is stored in the gall bladder and released into the upper intestine by hormonal action (secretin, enterocrinin) when food is present in the intestine.
5. **Enterohepatic circulation:** The bile acids help in the digestion and absorption of lipids in food. They are then acted upon by intestinal bacterial enzymes and then partly converted to secondary bile acids—deoxycholic acid (from cholic acid) and lithocholic acid (from chenodeoxycholic acid).

The bile acids are mostly reabsorbed in the intestine through the portal vein and then return to the liver. Of the total 15–30 g/day, of bile acids, 300–500 mg/day are excreted through feces, and the rest are reabsorbed.

Derangement of Bile Acid Metabolism

SAQ: Write a note on the derangement of bile acid metabolism.

Ans: The regulation of bile acid metabolism is a major function of the liver. Derangement in bile acid metabolism indicates liver dysfunction. Through an orderly process of bile acid synthesis, conjugation, and secretion, the liver serves to maintain cholesterol balance and intestinal absorption of lipids. Alterations in hepatic bile acid synthesis, intracellular metabolism, excretion, intestinal absorption, or plasma extraction are reflected in derangements in bile acid metabolism.

BILIRUBIN METABOLISM

Competency achievement: The student should be able to:

PA25.1: Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, distinguish between direct and indirect hyperbilirubinemia

LAQ: Describe bilirubin metabolism, enumerate the etiology and pathogenesis of jaundice, and distinguish between direct and indirect hyperbilirubinemia.

Ans: Refer to Ch 13, p 298

Q: What is the difference between total, direct, and indirect bilirubin?

Ans: Refer to Ch 13, p 298

Q: Write normal ranges of serum total bilirubin, direct bilirubin, and indirect bilirubin.

Ans: Prothrombin and heparin

Q: Write the definition of jaundice (Icturus).

Ans: Refer to Ch 13, p 298

SAQ: What is cholestasis? List two clinical conditions that cause extra-hepatic cholestasis.

Ans: Cholestasis means failure of the normal amount of bile to reach the intestine. It can be intra-hepatic, extra-hepatic, or due to both intra-hepatic and extra-hepatic.

Intra-hepatic cholestasis is caused by disturbed circulation of bile, due to liver cell inflammation.

Extra-hepatic cholestasis is caused due to obstruction to the flow of bile from the gall bladder to the small intestine. The following are the main reasons for extra-hepatic cholestasis:

1. Presence of gall stones in the bile duct.
2. A tumor of the bile duct.

LAQ: What is hyperbilirubinemia? Describe the pathophysiology, recommended laboratory tests, and expected test values in indirect hyperbilirubinemia (Pre-hepatic jaundice).

Ans. Refer to Ch 13, p 298

LIVER DISEASES

BAQ: Write a note on liver diseases.

Ans: Liver may get affected by pre-hepatic, hepatic, or post-hepatic conditions.

Pre-hepatic conditions are caused by the excessive formation of indirect bilirubin due to hemolytic anemia. Very high concentrations of indirect bilirubin may cause intra-hepatic cholestasis leading to disturbances in the bile pigment metabolism, leading to jaundice.

Hepatic conditions are caused mainly due to hepatitis A and hepatitis B viruses and also by hepatitis C, hepatitis D, hepatitis E, and Hepatitis G viruses. SARS-CoV-2 and parasites such as malarial parasites and amoebae also infect liver cells. Bacteria such as leptospira also infect the liver. Various drugs such as paracetamol, sulphonamides, anti-tuberculosis drugs, and alcohol also may cause drug-induced hepatitis.

Post-hepatic conditions are caused due to extra-hepatic cholestasis, by the gall stones in the bile duct, bile duct carcinoma, or due to bile duct stricture.

SAQ: Write a brief note on amebic hepatitis.

Ans: Amebic hepatitis is caused by the protozoa, *Entamoeba histolytica*. This parasite causes an intestinal infection which leads to amebic dysentery. Once intestinal infection takes place, the parasite may get carried by the bloodstream from the intestines to the liver and cause amebic hepatitis.

The symptoms are fever, pain, and extreme tenderness in the right hypochondrium. Liver scanning may confirm an amebic abscess. Moderate increase in serum bilirubin and SGPT levels are the main features of amebic hepatitis.

BAQ: Write a brief note on Weil's disease.

Ans: Weil's disease is a zoonotic bacterial disease of animals and humans. It is caused by the bacteria leptospira. Leptospira is transmitted from infected animals to humans through their urine, either directly or through infected soil or water, mainly during the rainy season due to clogged water. Weil's disease causes a self-limiting influenza-like illness, which may lead to more serious disease, if not treated in time. It also can progress to multiorgan failure leading to death.

Weil's disease can be diagnosed by a history of exposure to rats, severe pain in the back, a polymorphonuclear leukocytosis, and by demonstration of leptospiral specific antigen in the blood. The most striking biochemical finding in this condition is profound conjugated hyperbilirubinemia with bilirubinuria. SGPT and SGOT may be normal or slightly elevated, and a moderate increase in ALP is observed.

SAQ: Enumerate names of hepatotoxic drugs and their effects on the liver.

Ans: Examples of hepatotoxic drugs are: Tetracyclins, paracetamol, alcohol, etc.

These drugs may produce jaundice by a direct action on the liver cells, and the extent of the injury is dependent on the dose of the drug.

BAQ: Enumerate names of drugs that cause idiosyncratic reactions leading to hepatitis and cholestasis.

Ans: Idiosyncratic drug reactions are adverse drug reactions. These reactions do not occur in most patients at any dose and do not involve the known pharmacological properties of the drug. However, some individuals may get affected by idiosyncratic reactions.

1. The following drugs may produce hepatitis-like reactions:
   - Antituberculous drugs: Isoniazid, Rifampicin.
   - Anesthetic agents: Halothane
   - Sulfonamides
2. The following drugs may cause cholestatic reactions:
   - Oral contraceptives
   - Anabolic steroids (e.g. methandiaenone)
   - Antimicrobial agents: Para-aminosalicylic acid (PAS)
   - Tranquilizers (e.g. chlorpromazine)
   - Oral antidiabetic agents (e.g. chlorpropamide)

CLINICAL COURSE OF VITAL HEPATITIS

SAQ: Describe the clinical course of vital hepatitis.

Ans: The clinical course of viral hepatitis has two distinct phases: (1) Preicteric and (2) Icteric.

1. Preicteric phase lasts for 4–10 days. During this period, the bilirubin starts rising. This phase is manifested by nausea, vomiting, anorexia, abdominal discomfort, arthralgia, and skin rash.
2. The icteric phase is heralded by the appearance of jaundice. The liver is tender and palpable. Occasionally the spleen may be palpable. The icteric phase lasts for 1 to 4 weeks. During this phase, serum, bilirubin decreases and returns to normal.

SAQ: What are the symptoms of liver disease?

Ans: The symptoms of liver disease include loss of appetite, nausea, malaise, and fatigue. More specific symptoms include dark (yellow) urine, light stool, itching, abdominal pain (right upper quadrant discomfort), and bloating. Signs of advanced liver disease include weight loss, hepatomegaly, ascites, bruising and edema.

INVESTIGATIONS OF LIVER FUNCTIONS

LAQ: Describe and discuss liver disease and liver function tests (LFTs).

Ans: When the liver is diseased, one or more but not necessarily all, of its functions are impaired. There can be no test for liver functions as a whole. The various 'liver function tests' (LFTs) are tests of derangements of individual functions of the liver (or of similar derangements elsewhere in the body). Since many tests give similar abnormal results in a particular liver disease, it may be possible to extend a conclusion drawn from a single test.

The liver biopsy results may not be comparable with the LFTs since many functional changes are not mirrored by obvious structural changes in the liver cells.

The adult liver has a considerable functional reserve. An isolated part of the liver may be removed or severely damaged by a localized disease (may be carcinoma), and if the remainder is healthy, liver functions may remain normal. On the other hand, abnormal liver function test results may be obtained in a disease such as infective hepatitis, in which there is diffuse damage to the majority of liver cells.

Liver function tests may be used:

1. In the differential diagnosis of the different types of jaundice.
2. To assess the severity of liver damage in known liver disease.
3. To follow the trend of the disease.
4. To gauge post-operative risk.
5. To diagnose the presence of latent liver diseases, such as in the differential diagnosis of ascites or hematemesis.
6. To screen the suspected cases during the outbreak of infective hepatitis.
7. To screen the persons exposed to potential hepatotoxic materials in the industry.
8. To screen the persons exposed to hepatotoxic drugs to treat some other disease.

THE ROUTINELY PERFORMED LIVER FUNCTION TESTS (LFTs)

The routinely performed liver function tests (LFTs) are as follows:

These are based on the following disorders:

1. Abnormalities of bile pigments and bile salts excretion

   Tests

   a. Serum total, direct and indirect bilirubin.

   b. Urine bile salts, bile pigments, and urobilinogen.

2. Changes in certain enzymes

   Tests

   a. SGPT (ALT)

   b. SGOT (AST)

   c. Alkaline phosphatase (ALP) and, if necessary

3. Changes in plasma proteins

   a. Determination of total proteins, albumin globulin, and A/G ratio.

Clinical significance of serum total, direct and indirect bilirubin and urine bile salts, bile pigments, and urobilinogen

These tests are useful in the differentiation of pre-hepatic conditions from hepatic and post-hepatic conditions.

In pre-hepatic conditions, due to the increased breakdown of red blood cells in hemolysis the concentration of indirect bilirubin increases in the blood leading to prehepatic jaundice. It is only evident when serum total bilirubin exceeds 2.0 mg/dl concentration.

In urine examination, bile pigments are absent, since indirect bilirubin is water insoluble, which is very high, while direct bilirubin is normal and bile salts are not increased, however, very high levels of urobilinogen are detected.

Hepatic conditions are caused due to viral (hepatitis A, hepatitis B, Hepatitis C, etc) or bacterial infections and they can also be drug-induced (tetracyclines, paracetamol, alcohol, etc). Hepatic conditions may lead to jaundice. Clinically jaundice becomes apparent when the serum total bilirubin concentration exceeds 2 mg/dl. Both direct and indirect bilirubin may increase >1.0 mg/dl in hepatic conditions.

In urine examination, bile pigments are present, since direct bilirubin is >2 mg/dl, bile salts are increased, and high levels of urobilinogen are detected.

Post-hepatic conditions are caused due to extra-hepatic cholestasis, by the gall stones in the bile duct, bile duct carcinoma, or due to bile duct stricture, leading to extra-hepatic cholestasis. Post-hepatic conditions may lead to jaundice. Clinically jaundice becomes apparent when the serum total bilirubin concentration exceeds 2 mg/dl. Both direct and indirect bilirubin may increase >1.0 mg/dl in post-hepatic conditions.

In urine examination, bile pigments are present, since direct bilirubin is >2 mg/dl, bile salts are increased, and very high levels of urobilinogen are detected.

Clinical significance of Tests 1. SGPT 2. SGOT and 3. Alkaline phosphatase (ALP)

In pre-hepatic conditions, since, red blood cells contain SGOT and LDH, in hemolytic anemia, due to excessive hemolysis of red blood cells, plasma SGOT and LDH values increase above normal levels. Serum alkaline phosphatase is also normal if significant intra-hepatic cholestasis is not present.

ALP is excreted through the biliary system in the same manner as bilirubin is excreted via the bile ducts. Since the hepatobiliary system is not damaged in uncomplicated prehepatic (hemolytic) conditions, ALP values will be normal.

In hepatic conditions, since SGPT and SGOT are both present in liver cells, these are released into serum upon cell damage or destruction. The rise in SGPT and SGOT levels is related both to the rate and extent of liver cell necrosis. In viral hepatitis, very high levels are found. Peak values are usually reached within 12–14 hours of the onset of jaundice and values 10–20 times the upper limit of normal (upper limit of normal = 35 to 40 IU) are usual.

As the process of cell destruction declines in the recovery phase, the serum levels of transaminases also fall, usually reaching normal values within 25 days.

In hepatic conditions, due to disturbances in the hepatobiliary system caused by the swelling of the liver cells and also due to the blockage caused by bile thrombi to the free passage of bile, a moderate increase in ALP is observed.

In post-hepatic conditions, due to disturbances in the hepatobiliary system caused by the swelling of the liver cells and also due to the blockage caused by bile thrombi to the free passage of bile, a moderate increase in SGPT, SGOT is observed.

In post-hepatic conditions (extra-hepatic cholestasis), however, very high values of ALP (most of the times ten times the upper limit of normal) are observed. The rise in ALP usually parallels the degree of jaundice. After surgical removal of the cause of the obstruction, the enzyme levels slowly drop to normal.

Significance of Test 4: Determination of the serum proteins

The liver is the site of albumin and fibrinogen synthesis and some of the globulins, such as the alpha and beta globulin.

In pre-hepatic conditions, since liver cells are not affected significantly, normal levels of serum albumin and globulins are observed.

In hepatic conditions, in advanced liver disease, the albumin is decreased and globulins are often increased. Electrophoretic fractionation of serum proteins reveals that the increase is usually in gamma-globulin and also to a smaller extent in beta-globulin. Due to the alterations in serum proteins, the albumin/globulin (A/G) ratio is reversed. In infective hepatitis, quantitative estimation of albumin and globulin may give normal results in the early stages.

Normal values of the proteins are observed in post-hepatic conditions, at least so long as there is no complicating liver cell damage.

BAQ: Write expected blood test values of liver function tests in the Hepatic condition.

Ans: The following laboratory tests are performed on the serum and urine of a patient in hepatic as well as post-hepatic conditions:

- **Serum tests:** Total bilirubin, direct bilirubin, Indirect bilirubin
- **Urine tests:** Bile salts, bile pigments, urobilinogen

General laboratory observations in hepatic jaundice:

1. Serum: Icteric (yellow colored).
2. Serum tests:
   a. Total bilirubin : Increased
   b. Direct bilirubin : Increased
   c. Indirect bilirubin : Increased
   SGPT : High or very high
   SGOT : High or very high
   Alkaline phosphatase : Moderately increased
3. Urine:
   a. Freshly voided: Dark yellow colored
   b. On standing: Becomes dark due to the formation of high urobilin from increased urobilinogen
4. Urine tests:
   a. Bile salts : Present
   b. Bile pigments : Present
   c. Urobilinogen : Increased

BAQ: Write expected blood test values of liver function tests in post-hepatic conditions.

Ans: General laboratory observations in post-hepatic jaundice:

1. Serum: Icteric (yellow colored).
2. Serum tests:
   a. Total bilirubin: Increased
   b. Direct bilirubin: Increased
   c. Indirect bilirubin: Increased
   SGPT: High
   SGOT: High
   Alkaline phosphatase: Very high
3. Urine:
   a. Freshly voided: Dark yellow colored

  b. On standing: Becomes dark due to the formation of high urobilin from increased urobilinogen

4. Urine tests:
  a. Bile salts: Present
  b. Bile pigments: Present

Q: Write general laboratory observations in pre-hepatic conditions.

Ans: Refer to Ch:13, p299

Multiple Choice Questions

Q1. The amount of bilirubin forms from 1 g hemoglobin (at the end of RBC-life span) is about

A. 1.0 g B. 1.0 mg
C. 35 mg D. 3.5 mg

Q2. Which of the following enzyme is responsible to convert indirect bilirubin into direct bilirubin in the liver?

A. SGPT
B. SGOT
C. Gamma GT
D. Glucuronyl transferase

Q3. Indirect bilirubin is soluble in all of the following *except*

A. Water B. Ethyl alcohol
C. Methyl alcohol D. Chloroform

Q4. Direct bilirubin is soluble in

A. Water
B. Methyl alcohol
C. Ethyl alcohol
D. All of the above

Q5. Bile acids are synthesized from

A. Triglycerides
B. Saturated fatty acids
C. Unsaturated fatty acids
D. Cholesterol

Q6. Primary bile acids are

A. Glycocholic acid
B. Deoxycholic acid
C. Taurocholic acid
D. A and C

Q7. Secondary bile acids are

A. Glycocholic acid B. Deoxycholic acid
C. Taurocholic acid D. Lithocholic acid

Q8. Following clinical conditions may lead to prehepatic jaundice, *except*

A. Thalassemia major
B. Infective hepatitis
C. Sickle cell anemia
D. Hereditary spherocytosis

Q9. Following clinical conditions may lead to hepatic jaundice, *except*

A. Sickle cell anemia
B. Hepatitis A
C. Amebic hepatitis
D. Leptospirosis

Q10. Which drug interferes with the conjugation of bilirubin?

A. Rifampicin B. Aspirin
C. Primaquin D. Paracetamol

Q11. Mode of the spread of hepatitis A is due to

A. Infected blood transfusion
B. Injections of infected gamma globulin
C. Infected razor
D. Contaminated water or food

Q12. Mode of the spread of hepatitis B is due to

A. Infected blood transfusion
B. Contaminated injections
C. Contaminated water or food
D. A and B

Q13. Weil's disease is caused by

A. Hepatitis A B. Leptospira
C. Ameba D. Hepatitis D

Q14. Which of the following drugs are hepatotoxic?

A. Paracetamol
B. Tetracyclines
C. Tranquilizers
D. A and B

Q15. Drug that causes cholestatic reactions is

A. Paracetamol
B. Aspirin
C. Anabolic steroid
D. Tetracyclin

Q16. Very high indirect bilirubin, normal direct bilirubin, normal SGPT, and serum alkaline phosphatase indicate

A. Prehepatic condition
B. Hepatic condition
C. Posthepatic condition
D. B or C

Q17. High total bilirubin, indirect bilirubin, direct bilirubin, highly elevated SGPT, SGOT, and moderately elevated serum alkaline phosphatase indicates

A. Prehepatic condition
B. Hepatic condition
C. Posthepatic condition
D. Hepatitis A

Q18. High total bilirubin, indirect bilirubin, direct bilirubin, moderately increased SGPT, SGOT, and very high serum alkaline phosphatase indicates

A. Prehepatic condition
B. Hepatic condition
C. Posthepatic condition
D. Cirrhosis of liver

Q19. Jaundice indicates that total serum bilirubin is

A. > 2.0 mg/dl
B. < 1.0 mg/dl
C. All indirect type
D. All direct type

Q20. When bile salts and bile pigments are not detected in urine and the presence of high urobilinogen indicates

A. Prehepatic condition
B. Hepatic condition
C. Posthepatic condition
D. B or C

Q21. A 34-year-old man presented with a history of anorexia, nausea, pain in the right hypochondrium, and mild fever. His urine was dark yellow over the past three days. The most likely laboratory tests requested will be for

A. Diagnosis of Jaundice
B. Diagnosis and differentiation of jaundice
C. Diagnosis of renal disease
D. Diagnosis of infective hepatitis

Q22. A 67-year-old man presented with a history of anorexia, nausea, pruritus, and purpura. His urine was dark yellow over the past few days. The serum bilirubin test indicated that he was suffering from jaundice. To differentiate jaundice which of the following "enzyme panel tests" were required?

A. SGPT, SGOT, alkaline phosphatase
B. CPK, SGOT, LDH
C. Amylase, lipase, SGPT
D. Acid phosphatase, amylase, lipase

Answers

1. C **2.** D **3.** A **4.** D **5.** D **6.** D **7.** B **8.** B **9.** A **10.** C
11. D **12.** D **13.** B **14.** D **15.** C **16.** A **17.** B **18.** C **19.** A **20.** A
21. B **22.** A

Case Studies

Case 1: A 28-year-old man suffered from loss of appetite, nausea, mild fever, and pain in the right hypochondrium. On examination, it was found that the liver was palpable and tender. The colour of random urine was dark (yellow). His laboratory test reports were as follows:

	Reference range Normal values
Serum total bilirubin: 5.9 mg/dl	Up to 1.0 mg/dl
Serum direct bilirubin: 3.3 mg/dl	Up to 0.5 mg/dl
Serum indirect bilirubin: 2.5 mg/dl	Up to 0.5 mg/dl
SGPT: 550 IU	5–35 IU
SGOT: 365 IU	8–40 IU
	Reference range Normal values
Alkaline phosphatase (ALP): 110 IU	20–80 IU
Urine bile pigments: Present, ++	Absent
Urine bile salts: Present	Absent
Urine urobilinogen: Present, +	Normal

1. What is the probable diagnosis?

Ans: Hepatic jaundice (hepatitis, hepatic condition), since, very high values of serum total bilirubin, direct bilirubin, indirect bilirubin, and presence of bile salts, bile pigments with increased urobilinogen in urine indicated that the patient was suffering from jaundice (serum total bilirubin >2.0 mg/dl). The type of jaundice is hepatic**,** since very high values of SGPT, SGOT, and a moderate increase in ALP indicate hepatocellular damage.

2. What is the biochemical basis for the increase in total bilirubin (serum direct and indirect bilirubin)?

Ans: Hepatitis leads to disturbance in the excretion of bile in the intestine. Hence serum total bilirubin increases in the blood leading to jaundice.

3. What is the biochemical basis for the increase in serum SGPT, SGOT, and serum alkaline phosphatase?

Ans: Hepatitis leads to necrosis of liver cells, which are rich in SGPT and SGOT. Serum ALP is an excretory enzyme in bile, and due to disturbed excretion of bile, the level of serum ALP increases.

4. Why the color of the urine of this patient was dark yellow?

Ans: Due to the presence of excretory high bile pigments (bilirubin) in blood, when blood is filtered at the glomeruli, water-soluble bilirubin is excreted in the urine, which is yellow.

5. What is the probable line of treatment?

Ans: It is necessary to find out the **cause of the hepatic condition** and then according to the cause of the disease treatment is given. Hepatic jaundice may be caused due to viral or bacterial infections, or due to toxic effects of drugs or chemicals.

BAQ: Show horizontal integration of symptoms and test reports of this patient with anatomy, physiology, and nutrition.

Ans:

Horizontal integration with anatomy

Inflammation of liver tissue

Horizontal integration with physiology

Disturbed bile pigment and bile salt metabolism

Horizontal integration with nutrition

Prescription of a diet low in lipids and controlled intake of proteins and vitamins.

BAQ: Show vertical integration of symptoms and test reports of this patient with General medicine and pathology.

Ans:

Vertical integration with general medicine

Study of hypothyroidism

Vertical integration with pathology

Study of cause, magnitude of hepatic damage and prognosis of hepatitis.

Case 2: A 63-year-old man presented with jaundice. There was no history of injections or transfusions. He did not drink alcohol. He complained of intense pruritus during the past 2–3 months. His laboratory test reports were as follows:

	Reference range Normal values
Serum total bilirubin: 12.9 mg/dl	Up to 1.0 mg/dl
Serum direct bilirubin: 7.3 mg/dl	Up to 0.5 mg/dl
Serum indirect bilirubin: 5.6 mg/dl	Up to 0.5 mg/dl
SGPT: 750 IU	5–35 IU
SGOT: 1130 IU	8–40 IU
Alkaline phosphatase: 458 IU	20–80 IU
Urine bile pigments: Present, ++	Absent
Urine bile salts: Present	Absent
Urine urobilinogen: Present, increased	Normal

1. What is the probable diagnosis?

Ans: Post-hepatic condition (obstructive jaundice), since very high values of serum total bilirubin, direct bilirubin, indirect bilirubin, presence of bile salts, bile pigments with increased urobilinogen in urine indicated that the patient was suffering from jaundice (serum total bilirubin >2.0 mg/dl). Moderate increase in SGPT and SGOT, and very high values of serum alkaline phosphatase indicate obstructive jaundice.

2. What is the biochemical basis for an increase in total bilirubin (serum direct and indirect bilirubin) and pruritis?

Ans: Obstructive jaundice leads to disturbance in the excretion of bile in the intestine. Increased bile salts in blood circulation lead to pruritus.

3. What is the biochemical basis for the increase in serum SGPT, SGOT, and serum ALP?

Ans: Intra-hepatic and extra-hepatic cholestasis leads to necrosis of liver cells, due to poor supply of blood to liver cells. The increase in SGPT and SGOT is because of necrosis of liver cells which are rich in SGPT and SGOT. Serum ALP is an excretory enzyme in bile and due to obstruction to the excretion of bile [failure of a normal amount of bile to reach the intestine (cholestasis)], it increases significantly.

4. What is the probable line of treatment?

Ans: It is necessary to find out the cause of the post-hepatic condition that leads to the obstruction of the flow of bile in the small intestine. Ultrasound sonography gives an idea of specific obstruction and according to that the line of treatment is decided.

BAQ: Show horizontal integration of symptoms and test reports Case 2 with anatomy, physiology and nutrition.

Ans:

Horizontal integration with anatomy

Inflammation of liver tissue

Horizontal integration with physiology

Disturbed bile pigment and bile salt excretion and metabolism

Horizontal integration with nutrition

The suggestion of a diet low in lipids and a controlled intake of proteins and vitamins.

BAQ: Show vertical integration of symptoms and test reports of Case 2 with general medicine and pathology.

Ans:

Vertical integration with general medicine

Study and management of post-hepatic diseases

Vertical integration with pathology

Study of causes and prognosis of the post-hepatic condition.

Case 3: A 14-year-old boy presented with significant loss of weight, severe anemia, jaundice, and chronic ulcers on internal and external malleoli. His random urine examination report indicated the absence of bile salts and bile pigments and the presence of a high concentration of urobilinogen (++++). His blood examination reports were as follows:

	Reference range (Normal values)
Serum total bilirubin: 9.0 mg/dl	Up to 1.0 mg/dl
Serum direct bilirubin: 0.6 mg/dl	Up to 0.5 mg/dl
Serum indirect bilirubin: 8.4 mg/dl	Up to 0.5 mg/dl
SGPT: 30 IU	5–35 IU
SGOT: 68 IU	8–40 IU
Alkaline phosphatase: 65 IU	20–80 IU

1. What is the probable diagnosis?

Ans: Prehepatic condition, since high serum total bilirubin, normal direct bilirubin, and very high indirect bilirubin indicate **hemolytic jaundice.**

2. What is the biochemical basis for an increase in total bilirubin and indirect bilirubin?

Ans: The patient is anemic. Excessive destruction of red blood cells takes place in hemolytic disease, due to decreased life of RBCs.

Bilirubin is the excretory end product of protoporphyrin (separated from destroyed RBCs) metabolism. High levels of bilirubin form from high levels of protoporphyrin. The liver is unable to excrete very high levels of bilirubin. Hence total and indirect bilirubin rise in the blood.

3. What is the biochemical basis for normal serum SGPT and serum alkaline phosphatase?

Ans: Since liver cells are not affected, SGPT and SGOT values are normal. Serum ALP is an excretory enzyme in bile, and excretion of bile is normal, hence normal serum ALP value.

4. Why urobilinogen level has increased in urine?

Ans: Urobilinogen is the end product of bile pigment metabolism. Since serum bilirubin is very high, urobilinogen also increases circulation.

5. What is the mechanism behind chronic ulcers on internal and external malleoli?

Ans: Due to the excessive destruction of red blood cells, released high quantity of hemoglobin deposits in the skin.

6. What is the probable line of treatment?

Ans: It is necessary to find out the cause of hemolytic anemia. It may be primary (congenital) or acquired hemolytic anemia. According to the cause of the disease, treatment is given. Treatment for primary hemolytic anemia is supportive, while acquired hemolytic anemia can be treated.

BAQ: Show horizontal integration of symptoms and test reports of Case 3 with anatomy and physiology.

Ans: Horizontal integration with anatomy

Excessive destruction of red blood cells

Horizontal integration with physiology

Disturbed bilirubin metabolism

BAQ: Show vertical integration of symptoms and test reports of Case 3 with general medicine and pathology.

Ans: Vertical integration with general medicine

Study and management of hemolytic anemia

Vertical integration with pathology

Study of cause and prognosis of hemolytic jaundice

THYROID FUNCTION TESTS

Competency achievement: The student should be able to:

PY8.4: Describe function tests of the thyroid gland, adrenal cortex, adrenal medulla, and pancreas

SYNTHESIS OF THYROID HORMONES

BAQ: Write a note on the synthesis of thyroid hormones.

Ans: Refer to Ch: 8, page 202

BAQ: Write a note on the feedback system between the hypothalamus, pituitary gland, and thyroid gland and its significance.

Ans: The functions of the thyroid gland, the hypothalamus, and the pituitary gland are closely integrated. A tightly controlled feedback system exists between these glands so that thyroid hormone concentrations in blood are maintained within normal limits.

From the schematic outline shown (Fig. 17.1), it can be seen that TRH is produced by the hypothalamus and travels by way of a portal blood system to the pituitary. There, this hormone mediates the release of TSH. The effect of TRH on the pituitary is influenced by free T4 returning to the pituitary from the bloodstream. Excessive levels of free T4 depress TSH release from the pituitary, and low levels increase TSH release. TSH, entering the bloodstream from the pituitary, then causes the thyroid to trap organic iodine and secrete thyroid hormones (Fig. 17.1).

TSH controls the size and number of thyroid follicular cells. A rise in the thyroid hormone concentration exerts an inhibitory effect on the pituitary response to TRH (negative feedback). Similarly, a decrease in thyroid hormone concentration causes an increase in both TRH and TSH secretion (positive feedback).

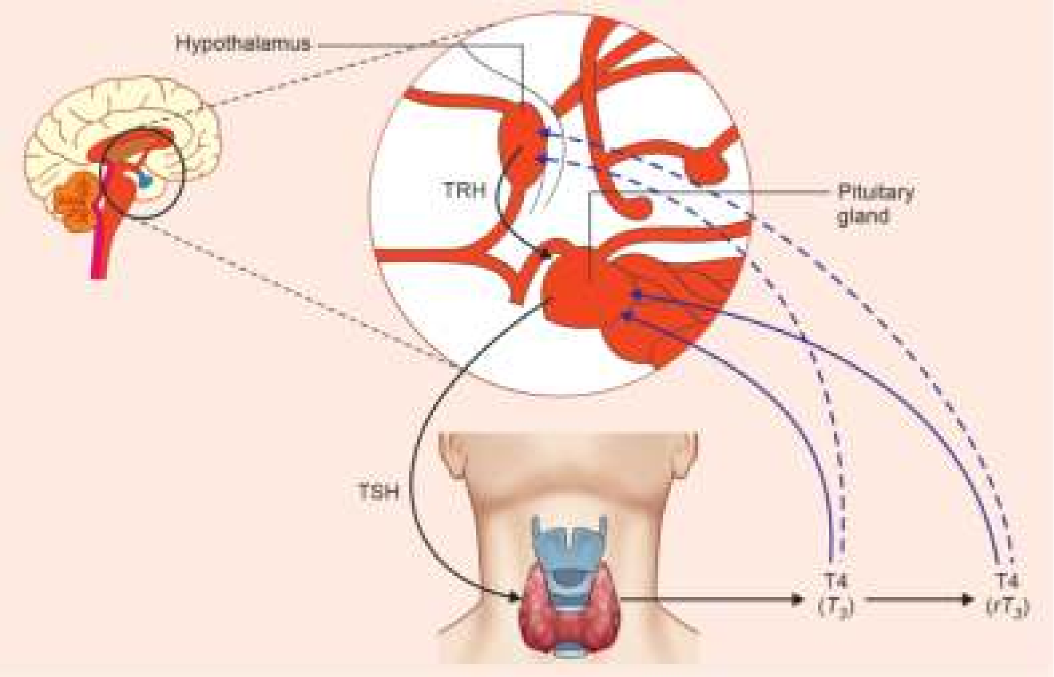


Fig. 17.1: Feedback system between the hypothalamus, pituitary gland, and thyroid gland

THYROID FUNCTIONS

BAQ: Write a note on thyroid functions.

Ans: The following are various functions of thyroid hormone:

1. Control of the basal metabolic rate and calorigenesis. This is controlled through increased oxygen consumption in tissue via the effects of thyroid hormone on membrane transport, increased mitochondrial metabolism by the cycling of Na^+/K^+-ATPase, and increased synthesis and consumption of ATP.
2. Essential for neural development, normal growth, and sexual maturation in mammals.
3. Stimulation of carbohydrate metabolism, protein synthesis, metabolism of cholesterol, triglycerides, calcium, and inorganic phosphorus.
4. Increases sensitivity of adrenergic receptors to catecholamines and controls heart rate and myocardial contractility.

THYROID DISEASES

BAQ: Write a note on goiter.

Ans: An enlarged thyroid gland, with or without nodules, is called a goiter. A nodule is the enlargement of a part of the gland. Multiple nodules may be present in the case of goiter, and they may be as much as 100 times the size of the normal thyroid gland. Any such region of the thyroid should be tested for cancer (by ultrasound and scanning techniques). Goiter can be simple or toxic.

Simple goiter: In this condition, there is an enlargement of the thyroid gland and deficient secretion of T3 and T4 despite elevated TRH and TSH. There is diffuse or nodular hyperplasia of the gland. Sometimes the extra thyroid tissue can maintain normal hormone levels.

Toxic goiter (hyperthyroidism): Toxic goiter (hyperthyroidism) is defined as a hypermetabolic condition caused by the excessive production of thyroid hormones. Hyperthyroidism can be primary or secondary:

- *Primary hyperthyroidism* is the term used when the levels of thyroid hormones T3 and T4 increase due to a disease of the thyroid gland.
- *Secondary hyperthyroidism* is the term used when the thyroid gland is stimulated by excessive thyroid-stimulating hormone (TSH) in the circulation due to a pituitary tumor or an autoimmune disorder; leading to the development of an IgG antibody against the thyroid TSH receptor. This results in the overproduction of T3 and T4, as observed in Graves' disease. The most common cause of hyperthyroidism is Graves' disease.

SAQ: What are the symptoms of hyperthyroidism?

Ans: The following are symptoms of hyperthyroidism:

Enlargement of the thyroid gland, cardinal arrhythmia, loss of weight, muscle wasting, weakness, physical restlessness, mental excitability, decreased fertility, menstrual abnormalities, malabsorption, increased gastrointestinal mobility, and pregnancy loss.

BAQ: Write a note on hypothyroidism and related symptoms.

Ans: Hypothyroidism is a deficiency of thyroid hormone secretion and action. Both sexes are affected more frequently with increasing age, and women suffer from hypothyroidism more often than men. Commonly observed clinical symptoms of hypothyroidism are fatigue, lethargy, and cold intolerance. Hypothyroidism can be primary or secondary.

Primary hypothyroidism: Primary hypothyroidism is caused due to failure of the thyroid gland to synthesize normal quantities of thyroid hormones (T3 and T4). The main reasons for primary hypothyroidism are:

1. **Iodine deficiency:** Iodine deficiency is the most common cause of hypothyroidism.
2. **Presence of antithyroid antibodies:** Due to the presence of antithyroid antibodies, the synthesis of T3 and T4 is impaired.
3. **Viral or bacterial infections:** Viral or bacterial infection of the thyroid gland may decrease the normal synthesis of T3 and T4.
4. **An inherited defect:** An inherited defect in thyroid hormone biosynthesis may lead to a decrease in the normal synthesis of T3 and T4.

Decreased levels of T3 and T4 and elevated levels of TSH are useful in the early detection of primary hypothyroidism. Primary hypothyroidism can be treated well by the daily administration of oral thyroxine.

Secondary hypothyroidism: Secondary hypothyroidism occurs as a result of hypothalamic or pituitary diseases that produce a deficiency in either TSH, TRH, or both.

Myxedema develops when a deficiency of T3 and T4 occurs after normal mental and physical development is complete. The underlying causes of simple goiter are:

1. Persistent iodine deficiency
2. Genetic abnormality
3. Interference of certain drugs and chemicals such as para-aminosalicylic acid, sulfonylureas, resorcinol, and anti-thyrotoxicosis drug.

Symptoms of hypothyroidism

Ans: Depression, decreased concentration, decreased heart rate, increased blood pressure, decreased cardiac output, decreased kidney function, edema, constipation, decreased gastrointestinal mobility, decreased fertility, menstrual abnormalities, and hyperlipidemia.

BAQ: Write a note on myxedema.

Ans: Myxedema is a severe form of hypothyroidism, as well as hyperthyroidism. It leads to the accumulation of mucopolysaccharides in the skin and other tissues, leading to the increased thickness of the skin and the thickening of facial features.

Myxedema term can be used both in the context of hyperthyroid states and hypothyroid states. In hypothyroidism, the term myxedema is also used to describe the clinical syndrome secondary to hypothyroidism. Symptoms can include mental slowness, weakness, depression, bradycardia, fatigue, hypothermia, alopecia, etc.

In hyperthyroidism, myxedema typically presents in specific areas and is exhibited as pretibial myxedema (infiltrative dermopathy) and exophthalmos (protruding eyeballs). It is related to high levels of TSH receptor stimulation and inflammation mounted against the TSH receptor itself.

SAQ: What is autoimmune thyroiditis?

Ans: In autoimmune thyroiditis, there is autoimmunity to T3, T4, thyroglobulin, and thyroid gland cells. The antibodies prevent the synthesis and release of hormones, causing myxedema. The variants of this disease are Hashimoto's disease, primary myxedema, and focal thyroiditis.

BAQ: Write a note on Hashimoto's disease.

Ans: Hashimoto's disease is an autoimmune disorder, in which, the immune system creates antibodies that attack thyroid cells, leading to a significant decrease in secretions of T3 and T4. The following factors are responsible for the onset of Hashimoto's disease:

1. Genetic factors
2. Stress
3. Environmental factors such as infections
4. Radiation exposure

The following factors are associated with an increased risk of Hashimoto's disease:

- **Sex:** More women suffer from Hashimoto's disease, compared to men.
- **Presence of other autoimmune diseases:** Other autoimmune diseases such as type-1 diabetes mellitus, rheumatoid arthritis, etc. increase the risk of developing Hashimoto's disease.
- **Pregnancy:** Specific changes in immune function during pregnancy may lead to Hashimoto's disease, after pregnancy.
- **Excessive iodine intake:** High intake of iodine in the diet may trigger a risk for Hashimoto's disease.

SAQ: Write one common feature and one difference between Graves' disease and Hashimoto's disease.

Ans: Common feature: Antibodies against specific components of the thyroid gland are responsible for both Graves' disease and Hashimoto's disease.

Difference: In Graves' disease, specific antibodies act on TSH receptors and are responsible for the secretion of very high levels of T3 and T4.

In Hashimoto's disease, specific antibodies act against globulin or peroxidase in the thyroid gland or on cells of the thyroid tissue. As a result, the thyroid gland is unable to secrete normal levels of T3 and T4 leading to hypothyroidism.

BAQ: Write a note on hyperthyroidism and related symptoms.

Ans: Hyperthyroidism is defined as a hypermetabolic condition caused by the excessive production of thyroid hormones. Women are more prone to develop hyperthyroidism than men. Hyperthyroidism can be primary or secondary.

Primary hyperthyroidism is the term used when the levels of thyroid hormones T3 and T4 increase due to a disease of the thyroid gland.

Secondary hyperthyroidism is the term used when the thyroid gland is stimulated by excessive thyroid-stimulating hormone (TSH) in the circulation due to a pituitary tumor.

The most common cause of hyperthyroidism is Graves' disease. It is an autoimmune disorder, and its etiology involves the development of an IgG antibody against the thyroid TSH receptor. This results in the overproduction of T3 and T4.

Excessive secretions of T3 and T4 also result from autonomous production from toxic solitary adenoma, from solitary or multiple thyroid nodules.

Inflammatory changes in the thyroid tissue due to bacterial or viral infections (subacute thyroiditis) may lead to leakage of thyroid hormones.

Some secondary causes of hyperthyroidism include excess iodine ingestion, thyroid carcinoma, exogenous intake of thyroid hormone, and drug-induced thyrotoxicosis with medications such as amiodarone (an antiarrhythmic drug). The main effects of hyperthyroidism are due to increased metabolic rate (MR).

The sustained elevated levels of T3 and T4 cause:

- Cardinal arrhythmia, since the heart tries to supply extra oxygen and nutrition to the hyperactive blood cells.
- Increased gluconeogenesis from body protein to provide extra energy. This leads to loss of weight, muscle wasting, and weakness.
- Decreased glucose tolerance leading to hyperglycemia.
- Excessive heat production due to increased MR.
- Physical restlessness and mental excitability due to neuron hyperactivity.
- Enlargement of the thyroid gland, which may develop single or multiple hormone-secreting nodules or diffuse hyperplasia or secretory cells as in Graves' disease and toxic nodular goiter.
- Increased renal blood flow and glomerulus filtration rate (GFR).
- Decreased fertility, menstrual abnormalities, and pregnancy loss.
- Malabsorption and increased gastrointestinal mobility.
- Hepatic dysfunction, negative protein balance, and increased lipid degradation.

The various symptoms of hyperthyroidism include:

1. Nervousness
2. Fatigability
3. Excessive sweating
4. Loss of weight
5. Increased body temperature
6. Increase in heart rate
7. Characteristic protrusion of the eyeballs, etc. Hyperthyroidism can be treated by surgery or by antithyroid drugs (goitrogens).

BAQ: What is euthyroid sick syndrome?

Ans: Euthyroid sick syndrome is associated with thyroid hormone deficiency or excess in the absence of definable thyroid tissue. Clinical conditions related to non-thyroid illness are observed by alterations in the concentration of thyroid-binding proteins, due to peripheral resistance to thyroid hormone or by the actions of certain drugs.

A euthyroid sick syndrome is a state of adaptation or dysregulation of thyrotropic feedback control where the levels of T3 and/or T4 are at unusual levels, but the thyroid gland does not appear to be dysfunctional. This condition is often seen in starvation or critical illness related to malignancy, heart failure, cirrhosis of the liver, chronic renal failure, diabetic ketoacidosis, trauma, hypothermia, and stress.

Laboratory Tests on Thyroid function and Related Clinical Significance

LAQ: Describe function tests of the thyroid gland and related clinical significance.

Ans: The following are the important thyroid function tests.

1. Determination of T3, T4 and TSH
2. Determination of free T4 (FT4)
3. Determination of free T3 (FT3)
4. Determination of TBG
5. Determination of TRH

Tests for autoimmune thyroid disease: In addition to the above-mentioned tests, the following tests are performed for the determination of specific antibodies:

A. Antithyroglobulin antibodies (TgAb)

B. Antithyroid peroxidase antibodies (TPOAb)

C. TSH receptor antibodies

Clinical significance:

- Elevated T3 and T4 and decreased TSH are seen in primary hyperthyroidism. While decreased T3 and T4 and elevated TSH are seen in primary hypothyroidism.
- In secondary and tertiary hypothyroidism (decreased TSH and decreased TRH, respectively), low values of T3 and T4 are observed due to pituitary or hypothalamic lesions. In these conditions, TSH is suppressed to a subnormal level.
- T3 and T4 levels are also elevated in conditions in which TBG levels are high, as in hyperproteinemia, pregnancy, and during estrogen therapy.
- Low T3 and T4 levels are also decreased in those conditions in which TBG values are low. These clinical conditions are—nephritis, severe hepatitis, gastrointestinal disorders, and patients undergoing androgen, testosterone, or anabolic steroid therapy.
- Determination of TRH is used to differentiate the primary, secondary, and tertiary types of hypothyroidism and primary hyperthyroidism (when elevated T_3 and T_4 are observed with low levels of TSH). TSH is measured after the administration of exogenous TRH to assess the response of the anterior pituitary gland. The following are specific responses:
  1. Secondary hypothyroidism (anterior pituitary failure): No response.
  2. Tertiary hypothyroidism: (hypothalamic failure): TSH rises after a delay.
  3. Hyperthyroidism: Very slight or no response.

Multiple Choice Questions

Q1. Excess secretion of T3 and T4 hormones is observed in

A. Graves' disease
B. Hashimoto's disease
C. Addison's disease
D. Cushing syndrome

Q2. In primary hypothyroidism the level of which of the following hormones is increased?

A. T3 B. T4
C. TSH D. LH

Q3. Thyroid hormones are synthesized by the iodination of

A. Tyrosine B. Phenylalanine
C. Glycine D. Tryptophan

Q4. Basal metabolic rate (BMR) is increased in

A. Myxedema B. Addison's disease
C. Hyperthyroidism D. Diabetes insipidus

Q5. A 42-year-old woman presented with depression, menstrual abnormalities, constipation and decreased heart rate. These are the most appropriate initial laboratory tests required for diagnosis

A. Serum estrogen
B. Serum LH
C. Serum FSH
D. Thyroid function tests

Answers

1. A **2.** C **3.** A **4.** C **5.** D

Case Studies

Case 1

A 49-year-old female experienced depression, menstrual abnormalities, lack of concentration, bradycardia, and the latest history of constipation. Her physician referred her to a pathology laboratory for "thyroid function tests". Her blood reports were as follows:

Serum	Normal range
T_3: 68 ng/dl	86–187 ng/dl
T_4: 2.7 μg/dl	4.5–12.5 μg/dl
TSH: 12.9 μIU/ml	0.3–5 μIU/ml
Serum total cholesterol: 325 mg/dl	150–250 mg/dl

1. What is the probable diagnosis?

Ans: Primary hypothyroidism, since serum levels of T3 and T4 are significantly low and high values of serum TSH.

2. What is the biochemical basis behind the increase in serum total cholesterol?

Ans: In hypothyroidism, due to a considerable decrease in metabolic rate, normal circulation and excretion of serum cholesterol is decreased, leading to an increase in serum cholesterol.

3. What is the biochemical basis behind the decrease in T3 and T4?

Ans: Either iodine deficiency or the presence of antibodies against thyroid tissue proteins leads to a decrease in serum T3 and T4.

4. What is the biochemical basis behind an increase in serum TSH?

Ans: It is the response of the pituitary gland to increase TSH levels to increase T3 and T4 levels by the thyroid gland (adjusted feedback mechanism).

5. What is the probable line of treatment?

Ans: Thyroxine tablets are prescribed to maintain normal levels of serum T3 and T4.

BAQ: Show vertical integration of symptoms and test reports of Case 1 with anatomy, physiology, nutrition, and biochemistry.

Ans: Horizontal integration with Anatomy

Enlarged, hypoactive thyroid gland.

Horizontal integration with physiology

Decrease in basal metabolic rate (BMR)

Horizontal integration with nutrition

The suggestion of diet and salt to meet the required RDA of iodine (if iodine deficiency is suspected)

Horizontal integration with biochemistry

Decrease in the synthesis of thyroid hormones and related decreased metabolism of lipids, carbohydrates, proteins, and oxidative pathways.

Q: Show vertical integration of symptoms and test reports of Case 1 with general medicine, pharmacology, pathology, and preventive medicine.

Ans: Vertical integration with general medicine.

Study of hypothyroidism

Vertical integration with pathology

To study the cause, diagnosis and prognosis of hypothyroidism.

Vertical integration with preventive medicine

Preventive measures like diet and salt support sufficient iodine to meet RDA (if iodine deficiency is suspected).

Case 2: A 38-year-old executive presented with loss of weight, physical restlessness, mental excitability, and prominent eyes (exophthalmic myxedema). On examination, his doctor observed that the patient had a slightly enlarged thyroid gland. His "thyroid function test" results were as follows:

	Normal range
T_3: 246 ng/dl	86–187 ng/dl
T_4: 16.7 μg/dl	4.5–12.5 μg/dl
TSH: 0.11 μIU/ml	0.3–5 μIU/ml

1. What is the probable diagnosis?

Ans: Primary hyperthyroidism, since serum levels of T_3 and T_4 are significantly high and low values of serum TSH.

2. What is the biochemical basis behind an increase in T_3 and T_4?

Ans: Probably due to the presence of antibodies against TSH receptors that mimic TSH leading to an increase in serum T_3 and T_4.

3. What is the biochemical basis behind the decrease in serum TSH?

Ans: It is the response of the pituitary gland to decrease TSH level since there is an increase in T_3 and T_4 levels by the thyroid gland (adjusted feedback mechanism).

4. What is the biochemical basis behind exophthalmic myxedema (protruding eyeballs)?

Ans: Due to increased deposition of hyaluronic acid, mucopolysaccharides, and glycosaminoglycans in the tissue around the eyeballs.

5. What is the probable line of treatment?

Ans: Carbimazole tablets, decrease the synthesis of T3 and T4 in the thyroid gland. Other options are the use of radioactive iodine that reduce hyperactive cell of the thyroid gland and if necessary, surgery of the thyroid gland.

BAQ: Show vertical integration of symptoms and test reports of Case 2 with anatomy, physiology, and biochemistry.

Ans: Horizontal integration with anatomy
Hyperactive thyroid gland.

Horizontal integration with physiology
Increase in basal metabolic rate (BMR)

Horizontal integration with biochemistry
Increased synthesis of thyroid hormones and related increased metabolism of lipids, carbohydrates, proteins, and oxidative pathways.

Q: Show vertical integration of symptoms and test reports of Case 2 with general medicine, pharmacology, pathology, and preventive medicine.

Ans:

Vertical integration with general medicine.
Study of hyperthyroidism

Vertical integration with pathology
To study the cause, diagnosis and prognosis of hyperthyroidism.

Vertical integration with preventive medicine
Preventive measures like diet and salt support sufficient iodine to meet RDA (to prevent excess intake of salt).

THYROID NATIONAL PROGRAM

LAQ: Write a note on the thyroid national program in India.

Ans: Thyroid national program in India:
An epidemiology study conducted at eight sites in India, namely Mumbai, Bangalore, Chennai, Goa, Delhi, Ahmedabad, Hyderabad, and Kolkata revealed the overall prevalence of hypothyroidism as 10.95%; of which, only 7.48% of patients self-reported the condition. 3.47% of hypothyroid cases were previously undetected. Hence, this is an important public health concern and a challenge to the policymakers of India. The government of India and the Ministry of Health and Family Welfare has the central and primary role in the implementation of the following health programs related to thyroid specifically.

Newborn screening (NBS): NBS is a medical procedure where a newborn baby is screened within 72 h of birth for any disorders or diseases. Significantly increased serum TSH above the normal level and low free T4 below the normal level are considered diagnostic parameters for the diagnosis of hypothyroidism (an important cause of goiter).

Childhood hypothyroidism (CH) is one of the most common preventable causes of mental retardation in children. The first multicentric study screening above 1 lakh neonates born throughout India was launched by the Indian Council of Medical Research (ICMR), and the National Task Force Team on NBS at AIIMS New Delhi (2007–2012) reveal a much higher incidence of CH all over India at 1 in 1172, particularly in South Indian population (1 in 727).

Timely treatment is very important to effect adequate neurocognitive development during the critical first 3 years of life. If the treatment is started early, chances are there that better intelligence levels in children can be achieved later in life.

National guidelines for screening of hypothyroidism during pregnancy (2014): The government of India released the following National Guidelines for Screening Hypothyroidism during Pregnancy in the year 2014.

1. The strategy is, high-risk pregnant women attending antenatal care outpatient departments shall be screened for hypothyroidism at the first antenatal visit for early diagnosis and treatment.
2. Patients coming from the periphery may be followed up by the obstetrician or physician or medical officer at their concerned primary or secondary health facility and cases with associated medical and obstetric complications would be referred to

physicians or obstetricians at medical college or district hospital.

3. The effects of hypothyroidism on maternal and fetal well-being are well documented.

National iodine deficiency disorders control programme: Iodine deficiency is the most common cause of preventable mental retardation and brain damage in the world. Iodine deficiency disorders (IDDs) surveys conducted by the Central and State Health Directorates, ICMR, and medical institutes since the year 1950 have demonstrated that IDD is a public health problem in all states and union territories in India. Thus, no state and union uerritories are free from IDD. National Iodine Deficiency Disorders Control Programme (NIDDCP) is in operation since 1987. The current important objectives and components of NIDDCP are as follows:

1. Conduct surveys to assess the magnitude of the IDDs
2. Supply of iodized salt in place of common salt in regions where significant cases of hypothyroidism are indicated
3. Resurvey after every 5 years to assess the extent of IDDs and the impact of iodized salt.
4. Laboratory monitoring of iodized salt and urinary iodine excretion.

HORMONES OF THE ADRENAL CORTEX AND THEIR FUNCTIONS

SAQ: Enumerate the names of hormones of the adrenal cortex. From which organic compound these are formed?

Ans: The adrenal cortex produces three groups of hormones synthesized from cholesterol in the adrenal glands and gonads. They are collectively called corticosteroids. They are glucocorticoids, mineralocorticoids, and androgens (sex hormones).

SAQ: Write a short note on the synthesis of corticosteroids.

Ans: The corticosteroids are synthesized from cholesterol in the adrenal glands and gonads (Fig. 17.2). Cholesterol for the synthesis of corticosteroids is made available from the circulation in the form of low-density lipoprotein (LDL). Specific cell surface LDL receptors on the adrenal gland surface internalize the cholesterol, which is used as a substrate for the synthesis of corticosteroids. The nature and quantity of steroid hormones produced by gonads and the adrenal glands are different.

The glucocorticoids and mineral corticosteroids are about 21 carbon compounds having a steroid nucleus. These are synthesized in the adrenal cortical cells from

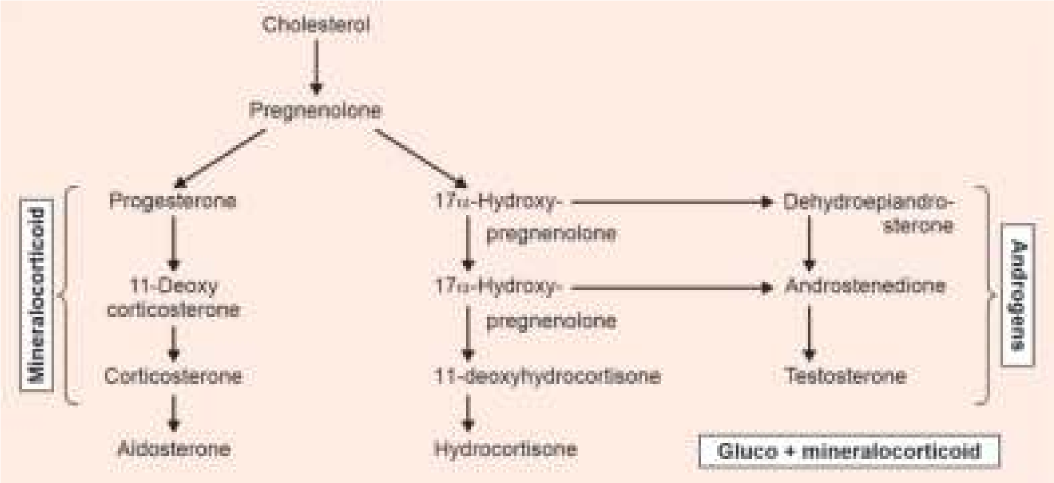

Cholesterol
Pregnenolone
Progesterone
11-Deoxy corticosterone
Corticosterone
Aldosterone
17α-Hydroxy-pregnenolone
17α-Hydroxy-pregnenolone
11-deoxyhydrocortisone
Hydrocortisone
Dehydroepiandro-sterone
Androstenedione
Testosterone
Mineralocorticoid
Androgens
Gluco + mineralocorticoid

Fig. 17.2: Synthesis of corticosteroids

cholesterol (Fig. 17.2). Cholesterol undergoes cleavage to form pregnenolone, which is a common precursor for the synthesis of all steroid hormones. This reaction is catalyzed by cytochrome p 450 side chain cleavage enzyme.

Adrenal steroidogenesis means the conversion of cholesterol to biologically active steroid hormones. It takes place under the influence of ACTH which makes more cholesterol available for conversion to pregnenolone and induces steroidogenic enzymes. The rate of release of corticosteroids is governed by the rate of biosynthesis.

Corticosteroids can also be locally synthesized in various other tissues, including skin, primary lymphoid organs, intestine and brain. Local corticosteroid synthesis is regulated through locally expressed mediators of the hypothalamic-pituitary-adrenal (HPA) axis or renin-angiotensin system (RAS). Locally synthesized glucocorticoids regulate the activation of immune cells, while locally synthesized mineralocorticoids regulate blood pressure and blood volume.

BAQ: What are glucocorticoids and their functions?

Ans: Glucocorticoids are secreted by the stimulation of ACTH from the anterior pituitary and also by stress. Cortisol and corticosterone are the main glucocorticoids. Cortisol is the major glucocorticoid synthesized from cholesterol in the zona fasciculate and fasciculata of the adrenal cortex. Cortisol has major effects on carbohydrate, lipid, and protein metabolism.

The main functions of glucocorticoids include the following:

1. Promotion of glycogenesis
2. Promotion of gluconeogenesis
3. Decrease in protein synthesis
4. Increase in lipolysis in adipose tissue
5. Promotion of reabsorption of sodium and water from the renal tubules.
6. Anti-inflammatory and immunosuppressive effects

BAQ: Write a note on the anti-inflammatory and immunosuppressive effects of glucocorticoids.

Ans: Glucocorticoid hormones have anti-inflammatory and immunosuppressive effects. Inhibition of the normal immune response results from the gradual destruction of lymphoid tissue. This follows by a decline in antibody production and a decrease in the number of lymphocytes, basophils, and eosinophils, eventually leading to a decrease in the number of T-lymphocytes.

The major suppressive effects of glucocorticoids on the immune system and inflammatory response is through the modulation of cytokine production. From immunocompetent cells, cytokines are released, which mediate both the acute and chronic phases of inflammation and participate in the control of the immune response. Glucocorticoids also have antiallergic properties.

BAQ: What are mineralocorticoids and their functions?

Ans: Aldosterone is the main mineral corticosteroid and it is synthesized exclusively in the zona glomerulosa region of the adrenal cortex from pregnenolone, which is an intermediate compound formed in the synthesis of steroids. Pregnenolone is converted to progesterone (Fig. 17.2). In two reactions, both catalyzed by 3-beta-hydroxysteroid dehydrogenase/isomerase. Progesterone is hydroxylated to form deoxycorticosterone, which is then converted to aldosterone.

Mineralocorticosteroids regulate salt homeostasis and extracellular fluid volume. It stimulates controlled reabsorption of sodium by the renal tubules and excretion of potassium. The amount of aldosterone produced is influenced by blood sodium levels. Corticosterone, cortisol, hydrocortisone (DOC), and 18-hydroxy-DOC are the other adrenocortical steroids that have mineralocorticoid properties with varying degrees of potency.

When renal blood flow is reduced, kidney cells secrete the enzyme, renin. This promotes the conversion of angiotensinogen (produced by the liver) to angiotensin, which stimulates the production of aldosterone by the adrenal cortex. By the action of aldosterone, the reabsorption of sodium and water excretion of potassium by the kidneys increases. This raises the blood volume and the flow of blood through the kidneys, suppressing renin production and aldosterone secretion.

BAQ: What are adrenal androgens and their functions?

Ans: The adrenal glands secrete estrogen, progesterone, and androgens, which are also produced by the gonads. 17α-hydroxypregnenolone is the precursor substrate for the synthesis of adrenal androgens and is produced in zona fasciculate and/or reticular. The primary adrenal androgens are dehydroepiandrosterone (DHEA) and androstenedione and also small amounts of testosterone and estrogens (estradiol, estrone, and insignificant amounts of progesterone) (Fig. 17.2).

Generally, males have greater amounts of muscle tissue than females due to higher amounts of androgens. These are known to promote the enlargement of skeletal muscle cells. Androgens do this by acting in a coordinated manner to enhance muscle functions by acting on many different types of cells.

Circulating androgens affect human behavior. Higher levels of androgens are associated with more aggression, energy levels, and drive to achieve goals. This happens because androgens influence human neurons by making them more sensitive to steroid hormones. Androgen levels are directly proportional to human aggression.

Sexual desire (libido) of the male is increased with androgens. Males have less adipose (fat) tissue than females. This is because androgens inhibit the ability of fat cells to store lipids.

BAQ: Write a note on the transport and excretion of corticosteroids.

Ans: The corticosteroids are inactivated in the liver by ring reduction to form tetrahydro derivatives. These are conjugated with glucuronic acid. Both free and conjugated corticosteroids are excreted into the intestine by way of the bile. Excretion of free and conjugated corticosteroids takes place by kidneys, after reabsorption in the intestine.

Androgens, mainly dehydroepiandrosterone and androsterone, are carried in the serum both in the free and conjugated form in association with the serum proteins. Most of the androgens are excreted into the urine as 17-ketosteroids.

The urinary neutral 17-ketosteroids are a reflection of the androgenic function of the subject. In the female, 17-ketosteroids are produced entirely by the adrenal cortex, and in the male, by the adrenal cortex and testes. 17-ketosteroids are elevated in adrenocortical carcinoma, bilateral hyperplasia of the cortex, and testicular tumors.

PATHOPHYSIOLOGY RELATED TO THE ADRENAL GLAND, ADDISON'S DISEASE

BAQ: Write a note on the pathophysiology of hypoadrenocorticism.

Ans: Hypoadrenocorticism means deficiency of aldosterone, secreted by the adrenal cortex. In humans, degeneration of the adrenal cortex results in Addison's disease. The effects of this disease include:

1. Excessive loss of sodium chloride in the urine
2. Elevated levels of potassium in serum.
3. Low blood pressure and low body temperature
4. Muscular weakness.

The following are other clinical conditions in which deficiency of aldosterone production occurs:

1. Inadequate production of renin by the kidneys leads to secondary aldosterone

deficiency (hyporeninemic hypoaldosteronism), in which aldosterone deficiency is accompanied by normal cortisol production.
2. Inherited enzyme defects in aldosterone biosynthesis.
3. Acquired forms of primary aldosterone deficiency (post-surgery and heparin therapy).

The following are the main symptoms of Addison's disease:

- Nausea
- Vomiting
- Loss of weight
- Loss of appetite
- Abdominal pain
- Extreme fatigue
- Low blood pressure
- Salt craving
- Hypoglycemia
- Appearance of patches of dark skin.

Cushing's Syndrome

BAQ: Write a note on the pathophysiology of hyperadrenocorticism.

Ans: Hyperadrenocorticism is caused by adrenocortical hyperplasia or benign or malignant tumors of the cortex, initiated by increased production of ACTH. This may lead to Cushing's disease. The effects of this clinical condition are:

1. Hyperglycemia and glycosuria.
2. Retention of sodium and water and excretion of potassium (hypokalemia).
3. Negative nitrogen balance, etc.

The main symptoms of Cushing's disease are as follows:

1. Weight gain in the face (moon face).
2. Increase in weight in the trunk, with thin legs and arms.
3. Appearance of a fatty lump between the shoulders (a buffalo hump).
4. Poor wound healing
5. Pink, purple stretch marks on the breasts, underarms, stomach, hips, and thighs.
6. Thin and frail skin
7. Easy skin bruising

BAQ: Write a note on cortisol.

Ans: Cortisol is a steroid hormone, in the glucocorticoid class of hormones. Cortisol is produced mainly by the zona fasciculata of the adrenal cortex in the adrenal gland. It is also produced in other tissues in very low quantities.

Ninety-five percent of cortisol in the blood is bound to transcortin (cortisol-binding protein). Hence the concentration of free cortisol is very small. Transcortin is almost fully saturated at normal cortisol concentration.

Plasma (or serum) cortisol concentration shows a diurnal variation. It is highest in the morning and lowest at night.

Blood for cortisol determination should be drawn between 0800 h (8 am) and 0900 h (9 am). Blood for cortisol determination can also be collected at 1600 h (4 pm) and 2300 h (11 pm) to detect loss of diurnal variation to diagnose early features of adrenal dysfunction (e.g. Cushing's syndrome).

The release of cortisol is increased in response to stress and low blood glucose concentration. In glucose metabolism, it stimulates gluconeogenesis, which leads to an increase in blood sugar. In lipid metabolism, the action of cortisol leads to lipogenesis. Prolonged action of cortisol may lead to proteolysis causing muscle wasting.

Cortisol plays a very important role in the metabolism of carbohydrates, fat, and protein. The action of cortisol decreases bone formation. Cortisol also acts to suppress the immune system. In the form of an immunosuppressive medication, it is used in the form of hydrocortisone and it has been used to decrease symptoms of acute respiratory distress syndrome.

BAQ: Write a note on the pituitary-adrenal relationship in Cushing's syndrome.

Ans: In the case of a normal individual, ACTH secreted by the pituitary gland stimulates the adrenal cortex to secrete cortisol, which exerts negative feedback on the release of ACTH by the pituitary. In Cushing's disease, ACTH secretion increases, and the pituitary is insensitive to feedback by normal levels of cortisol. However, higher levels of cortisol can produce negative feedback on ACTH secretion.

In the case of adrenal tumors, autonomous production of cortisol by the tumor cells inhibits ACTH secretion. In the case of "ectopic ACTH" secretion, high levels of ACTH secreted by the tumor stimulate excessive cortisol secretion, which leads to inhibition of ACTH by the pituitary.

BAQ: Write a note on Addison's disease (AD).

Ans: Addison's disease results from the progressive destruction of the adrenals. AD is relatively rare and may occur at any age, and affects both sexes equally. The most frequent primary cause of AD is idiopathic atrophy of adrenal glands due to autoimmune mechanisms, which lead to the deficiency of corticosteroids.

The secondary reason for AD is various diseases that may cause adrenal disease. These diseases are tuberculosis, cryptococcosis, coccidioidomycosis, and histoplasmosis which affect the adrenal glands.

Addison's disease is characterized by an insidious onset of weakness, fatigability, anorexia, nausea and vomiting, hypotension, occasional hypoglycemia, weight loss, and cutaneous and mucosal pigmentation.

In Addison's disease, low values of serum sodium, chloride, and bicarbonate are observed with an increase in serum potassium.

The diagnosis of adrenal insufficiency is made by ACTH stimulating test. In this test, cortisol response is observed 60 minutes after 250 mg of cosyntropin is given intravenously or intramuscularly. Cortisol values should exceed 495 nmol/L. Serum ACTH is usually elevated in primary adrenal insufficiency due to the loss of the cortisol-hypothalamic-pituitary feedback relationship.

In secondary adrenal insufficiency, low values of serum ACTH and cortisol are observed. By ACTH stimulation test, the observed values of serum ACTH are low with increased values of serum aldosterone.

All patients with adrenal insufficiency are treated with specific hormone replacement therapy.

CUSHING'S DISEASE

BAQ: Write a note on Cushing's disease.

Ans: Cushing's disease (syndrome) is caused mainly by pituitary corticotrope adenomas. Nearly 70% of patients are affected by pituitary corticotrope adenomas. Ectopic tumor ACTH secretion and adrenal adenomas, which secrete excessive cortisol, are responsible for the development of Cushing's disease.

Typical features of Cushing's disease are obesity, moon facies, hypertension, thin skin, decreased glucose tolerance, the appearance of acne, osteoporosis, hyperpigmentation, hypokalemia, etc.

The diagnosis of Cushing's syndrome is based on laboratory determinations of serum ACTH, cortisol, sodium, potassium, and chlorides. High levels of ACTH, cortisol, sodium, and chlorides and low levels of potassium are observed. Failure to suppress serum cortisol after an overnight 1mg dexamethasone suppression test is useful for identifying patients with hypercortisolism. A useful screening test is the determination of 24 hours excretion of free cortisol.

CONN'S SYNDROME

BAQ: Write a note on Conn's syndrome.

Ans: Conn's syndrome is associated with hypersecretion of aldosterone (the major mineralocorticoid). Hyperaldosteronism can be primary or secondary.

In primary hyperaldosteronism, excessive secretion of aldosterone originates within the adrenal gland. In secondary hyperaldosteronism, a stimulus outside the adrenal gland activates the renin-angiotensin system.

The controlled interaction of renin, angiotensin, and aldosterone is required in the regulation of extracellular fluid volume, the balance of sodium ions, potassium ions, and blood pressure. A change in one of these factors leads to a change in other factors.

Primary hyperaldosteronism is characterized by elevated plasma concentrations of aldosterone, alongs with hypertension and hypokalemia. Overproduction of aldosterone may be due to an adenoma of one adrenal gland, hyperplasia of aldosterone-producing cells in both the adrenal glands, and aldosterone-producing adrenal carcinoma.

In secondary hyperaldosteronism, the various events that disturb the normal equilibrium of renin release are as follows:

1. Sudden blood loss leads to a decrease in effective plasma volume, which leads to increased secretion of renin by the kidneys followed by increased angiotensin I and angiotensin II and an increased production of aldosterone by the adrenal glands. This results in the retention of water and sodium, an increase in extracellular volume, and a decrease in serum potassium.
2. Nephrotic syndrome, congestive heart failure, cirrhosis of the liver, etc, also may lead to secondary hyperaldosteronism.

BAQ: Write a note on the laboratory determinations of steroids.

Ans: Immunoassay techniques are used for the determination of steroids in body fluids. Similarly, other methods based on separation techniques are also used. These methods are liquid chromatography, gas chromatography, high-performance liquid chromatography, capillary electrophoresis, etc.

Steroids are measured in blood, urine, and saliva specimen.

Blood has been the preferred specimen for the determination of secretion rates of steroids from the related endocrine gland.

Urinary assays of steroids provide a good estimate of the secretory activity of the adrenal gland, although the urinary excretion of a hormone, or its metabolites (or both) does not account for the total secreted hormone from the gland.

Most steroids of clinical interest can be measured by using saliva. Determination of salivary steroids reflects the free (non-protein bound) steroid fraction in blood and may provide information similar to the urinary steroids.

HORMONES OF THE ADRENAL MEDULLA AND THEIR FUNCTIONS

LAQ: Write a note on the hormones of the adrenal medulla, their synthesis, functions, related pathophysiology and metabolism.

Ans: The hormones of the adrenal medulla are dopamine, norepinephrine, and epinephrine. They act as neurotransmitters and are required for adaptation to acute and chronic stress. Dopamine, serotonin, and norepinephrine are produced primarily in the regions of the medulla oblongata, pons, and midbrain (brainstem). These are produced by neurons with axons that project to defined and widespread areas of the brain or spinal cord. Although dopamine, serotonin and norepinephrine represent only 1% to 2% of the total neurotransmitters in the brain, all these serve very important roles in autonomic (involuntary), somatic (voluntary), and information processing functions.

The amines, dopamine, norepinephrine, and epinephrine are synthesized in the chromaffin cells of the adrenal medulla. The major product of the adrenal medulla is epinephrine, which is a catecholamine derivative of tyrosine and phenylalanine. It constitutes about 80% of the catecholamines in the medulla. The conversion of tyrosine to epinephrine takes place as shown in the following enzyme-catalyzed chemical reactions:

Tyrosine
↓ Tyrosine hydroxylase
Dihydroxyphenyl alanine (DOPA)
↓ DOPA decarboxylase
Dopamine

↓ Dopamine β-hydroxylase
Norepinephrine
↓ Phenylethanolamine-N methyl transferase (PNMT)
Epinephrine

Catecholamine biosynthesis is regulated at the tyrosine hydroxylase (TH) step. TH, present in the cytoplasm, is the rate-limiting enzyme. Stimulation of adrenergic nerves increases tyrosine hydroxylase activity. TH requires the presence of tetrahydrobiopterin, which is regenerated by reduced nicotinamide adenine dinucleotide.

The steps of the synthesis of catecholamines proceed in different cellular structures. Tyrosine penetrates the cytoplasm of neurons, where it is converted to dOPA. It is then converted to dopamine which penetrates storage vesicles, where it can be converted into noradrenaline by dopamine β-hydroxylase. Storage vesicles are smaller and denser than mitochondria and contain adenosine triphosphate (ATP), catecholamines (four catecholamine molecules for one molecule of ATP), calcium and magnesium, as well as proteins called chromogranin.

The adrenal medulla contains a higher amount of adrenaline than noradrenaline. Organs with sympathetic innervation, such as the heart and vessels, contain noradrenaline. These organs take up circulating catecholamines such as adrenaline from plasma. The sympathetic postsynaptic fibers synthesize norepinephrine, but not epinephrine. The brain is rich in noradrenaline and dopamine (extrapyramidal system) and contains a small quantity of adrenaline.

Like other transmitters, endogenous catecholamines are active only after their release. Under the effect of the nervous impulse, sympathetic terminations release norepinephrine; the adrenal medulla releases epinephrine and norepinephrine. This release is carried out by exocytosis, by breakage of storage vesicles in contact with the cellular membrane. As the adrenergic receptors are in contact with sympathetic terminations, norepinephrine released acts primarily on the organs where it is released and passes only secondarily in general circulation, whereas epinephrine and norepinephrine released by the adrenal medulla pass directly into the blood.

The plasma concentration of catecholamines results from their release into plasma, uptake by tissues and catabolism. In people lying at rest, plasma concentrations of dopamine, epinephrine, and noradrenaline are low (less than 0.1 μg/dl). However, their concentrations rise under physiological or pathological conditions. For example, when one goes from the supine to the standing position, noradrenaline concentration increases twofold. In patients suffering from a clinical condition such as pheochromocytoma, plasma catecholamines rise transiently and reach high levels.

Catecholamines cannot cross the blood–brain barrier. Hence these are synthesized in the brain locally. In certain central nervous system diseases, e.g. Parkinson's disease, there is a local deficiency of dopamine synthesis. L-dopa, the precursor of dopamine, readily crosses the blood–brain barrier and is an important agent in the treatment of Parkinson's disease.

Stimulation of the splanchnic nerve (which supplies the preganglionic fibers to the adrenal medulla) results in the exocytotic release of catecholamines. These circulate in plasma in a loose association with albumin. They have an extremely short biologic half-life (10–30 seconds). They act through two major classes of receptors. These are designated, α-adrenergic and β-adrenergic. Epinephrine binds to and activates both α- and β-receptors. Norepinephrine at physiologic concentrations primarily binds to α-receptors.

Catecholamines are rapidly metabolized by catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) to form inactive O-methylated and deaminated metabolites.

Metanephrine represents the methoxy derivatives of epinephrine and norepinephrine, while the O-methylated deaminated product of epinephrine and norepinephrine is vanillylmandelic acid (VMA).

The concentration of metanephrine or VMA in urine is elevated in more than 95% of patients with pheochromocytoma. Hence the determination of urine metanephrine or VMA is useful in the diagnosis of pheochromocytoma when coupled with the determination of plasma and urine catecholamines.

SAQ: Enumerate the action of epinephrin in a 'fight' or 'flight' situation after the initial sympathetic stimulation.

Ans: The following are the various effects of the action of epinephrin in a 'fight' or 'flight' situation after the initial sympathetic stimulation:

1. Constriction of skin blood vessels
2. Increase in metabolic rate
3. Dilation of blood vessels of muscle, heart, and brain
4. Dilation of the pupils
5. Dilation of the bronchioles, allowing an increase in air intake.

SAQ: What is the main function of noradrenalin?

Ans: The main function of noradrenalin is the maintenance of blood pressure by causing general vasoconstriction (except for the coronary arteries).

SAQ: What is the action of the drug metyrosine (demser), used to treat pheochromocytoma?

Ans: Metyrosine (demser) is an analog of tyrosine that inhibits tyrosine hydroxylase and decreases catecholamine stores. The drug is used to control high blood pressure in patients with pheochromocytoma.

SAQ: What is the clinical significance of the determination of plasma catecholamines and their metabolites? What laboratory tests are used for the laboratory determinations of plasma catecholamines and their metabolites?

Ans: Clinical laboratory determinations of plasma and urine catecholamines and their metabolites are important mainly in the diagnosis of catecholamine-secreting neuro chromaffin tumors such as pheochromocytomas, paragangliomas neuroblastoma, and serotonin-secreting carcinoids.

Laboratory Tests

1. Urinary and plasma catecholamines are determined by HPLC coupled with fluorometric determination.
2. 24 hours urine sample is used for the determination of VMA by spectrophotometric test.
3. Serum epinephrine, norepinephrine, and dopamine can be determined by radioimmunoassays (RIA), Enzymeimmunoassays (EIA), and HPLC methods.

BETA-BLOCKERS

BAQ: What are beta-blockers? Enumerate examples and their actions.

Ans: Beta-blockers are medications that reduce blood pressure by blocking the effects of epinephrine (adrenaline). Beta-blockers are used in combination with other modes of therapy and drugs for patients suffering from arrhythmia, chest pain, and specific heart-related ailments.

Effects of beta-blockers decrease heart beats which decrease blood pressure. Beta-blockers increase the diameters of veins and arteries to improve blood flow. Some beta-blockers affect mainly the heart and some beta-blockers affect both the heart and blood vessels. The choice of a specific beta-blocker can be decided according to a specific "case history".

Following are some examples of the beta blockers taken by mouth:

- Propranolol (Inderal)
- Bisoprolol (Zebeta)
- Butolol
- Acebutolol (tenormin)
- Metoprolol (lopressor)
- Madolol (corgard), etc.

Competency achievement: The student should be able to:

PY4.9: Discuss the physiological aspects of peptic ulcer, gastro-oesophageal reflux disease, vomiting, diarrhea, constipation, adynamic ileus, and Hirschsprung's disease

GASTRIC FUNCTION TESTS

Functions of Stomach

SAQ: Enumerate functions of the stomach.

Ans: The main function of the stomach is as an aid to digestion. The following are important gastric digestive functions:

1. The stomach acts as a reservoir for the storage of food for the initial digestion process. Due to the reservoir capacity of the stomach, its volume increases significantly, while internal pressure does not increase significantly.
2. Secretion of gastrin hormone by neuroendocrine G-cell of the stomach. Gastrin stimulates the parietal cells of the stomach to secrete hydrochloric acid (HCl).
3. HCl protects against invading pathogens and provides suitable acidic pH for the conversion of inactive pepsinogen to active pepsin.
4. The action of pepsin on proteins in food and the formation of intermediate digestive products, peptones, and proteoses.
5. An important role of the stomach in the mixing of stomach contents and initiation in gastrointestinal motility.
6. The stomach also plays an important motility role as a pump, which is anatomically provided by the distal two-thirds of the corpus, the antrum, and the pylorus.

BAQ: What are the chief constituents of gastric secretion and their respective functions?

Ans: The chief constituents of gastric secretion are:

1. Hydrochloric acid, secreted by the parietal cells
2. Pepsinogen, secreted by the chief cells
3. Renin
4. Intrinsic factor, and
5. Mucus.

The gastric secretion is produced in response to psychic factors (i.e., sight, taste, smell, etc.), by the action of gastrin hormone secreted by the gastric mucosa when food is present in the stomach. Insulin stimulates the vagus nerve by creating hypoglycemia, which increases the output of both acid and pepsin.

The various functions of gastric juice components are as follows:

1. HCl protects against invading pathogens and provides suitable acidic pH for the conversion of inactive pepsinogen to active pepsin.
2. Pepsinogen is activated to pepsin by gastric acid, which also provides the acid medium for the enzyme action on food proteins. The proteins present in food are partially broken down into peptones and proteoses.
3. Renin clots milk by converting milk protein caseinogen to casein.
4. The intrinsic factor (IF) is a glycoprotein produced by the parietal cells of the stomach. Intrinsic factor plays an important role in the transportation and absorption of vitamin B_{12} by the terminal ileum.
5. The intestinal mucus protects the stomach epithelium from bacteria by promoting their clearance and separating from the epithelial cells, which plays an important role in the inhibition of inflammation and infection.

SAQ: Describe the clinical condition hyperchlorhydria.

Ans: Hyperchlorhydria term is used when the maximum free acid exceeds 50 mEq/L. After the initial fall from a high fasting acidity, there may be a fairly rapid rise to concentrations exceeding 100 mEq/L. Such findings are seen occasionally in gastric ulcers and are most characteristic of duodenal ulcers. Blood, together with hyperchlorhydria, is suggestive of gastric ulcer and may also be found occasionally in gastric carcinoma. In pyloric stenosis, the volume of residual and resting contents may be very high with the presence of starch. A small percentage of cases of cancer of the stomach are found to have hyperchlorhydria.

SAQ: Describe the clinical condition achlorhydria.

Ans: Achlorhydria term is used when there is no secretion of hydrochloric acid. The enzyme pepsin may be present. When free acid and the enzyme both are absent, the term achylia gastrica is used. Achlorhydria is associated with persons belonging to older age groups, tuberculosis of the stomach, Addison's disease, ulcerative colitis, later stage of malignancy, and in pernicious anemia.

SAQ: What are gastrointestinal hormones? Name three gastrointestinal hormones.

Ans: Gastrointestinal hormones are polypeptides produced by mucosal endocrine cells of the stomach and small intestine. They are involved primarily in the regulation of the stomach, small intestine, pancreas, liver, and biliary tract. The major gastrointestinal hormones are gastrin, secretin, and cholecystokinin (pancreozymin).

SAQ: Write three functions of gastrin.

Ans: The following are the functions of gastrin:

1. It is the most effective activator of gastric acid secretion.
2. It causes chief cells to secrete pepsinogen, the zymogen (inactive) form of the digestive enzyme pepsin. It also stimulates intrinsic factor release from gastric mucosa.
3. It stimulates secretin release.

SAQ: What is Zollinger-Ellison syndrome?

Ans: Gastrin-producing pancreatic tumors cause Zollinger-Ellison syndrome. It is characterized by high levels of gastrin and elevated gastric acid secretion, leading to gastrointestinal ulcers.

SAQ: What is autoimmune gastritis?

Ans: In autoimmune gastritis, the immune system attacks the parietal cells leading to hypochlorhydria. This results in an elevated gastrin level in an attempt to compensate for increased pH in the stomach. Eventually, all the parietal cells are lost, and achlorhydria results leading to a loss of negative feedback on gastrin secretion.

SAQ: What is the reason for hyperacidity caused by *Helicobacter pylori* infection of the stomach?

Ans: The bacteria, *Helicobacter pylori*, secrete a large amount of urease enzyme, which acts on urea in the stomach to form ammonium carbonate, which has a neutralizing effect on stomach acid. To compensate for this effect, parietal cells of the stomach secrete excessive hydrochloride.

PANCREATIC FUNCTION TESTS

BAQ: Write a note on pancreatic function tests.

Ans: The following tests are pancreatic function tests, performed in a biochemistry laboratory:

Serum amylase, urine amylase, and serum lipase.

Clinical significance:

Determinations of serum and urine amylase are largely used in the diagnosis of diseases of the pancreas and the investigation of pancreatic function. In **acute pancreatitis**, a transient rise in serum amylase activity occurs within 2 to 12 hours of the onset. Serum amylase levels return to normal by the third or fourth day. Usually, high levels of amylase (four to six-fold elevations above the reference limit) are observed during 12 to 72 h. The parallel increase in serum lipase confirms acute pancreatitis. The elevation of serum amylase activity is reflected in the rise of urinary amylase activity. The urinary clearance of amylase is markedly increased in acute pancreatitis.

After an attack of acute pancreatitis, the serum lipase activity increases within 4 to 8 hours, peaks at about 24 hours, and decreases within 8 to 14 days. Serum lipase levels remain elevated longer than those of amylase. Lipase elevations usually parallel those of amylase but an increase in lipase activity may occur sooner or later than increases in amylase activity. Elevations between 2 and 50 times, the upper reference limit, have been reported.

Serum lipase assays may also be of value in the diagnosis of chronic pancreatitis and obstruction of the pancreatic duct by calculus or carcinoma of the pancreas.

Multiple Choice Questions

Q1. Cushing's syndrome leads to

A. Excessive cortisol secretion
B. Excessive thyroxine secretion
C. Decreased epinephrine secretion
D. Decreased cortisol secretion

Q2. Secretion of ACTH leads to an increase in

A. Cyclic AMP
B. Calcium ions
C. Cyclic GMP
D. Zinc ions

Q3. Hyperglycemic effect of glucocorticoids is due to

A. Inactivation of fructose 1,6-biphosphatase
B. Increase in gluconeogenesis
C. Increase in glycogenesis
D. Stimulation of synthesis of lipids

Q4. The hormone cortisol is synthesized in

A. Zona fasciculata
B. Chromaffin cells
C. Zona reticularis
D. Zona glomerulosa

Q5. All steroid hormones are formed from

A. Cholesterol
B. Pyrimidine
C. Glycerol
D. Purine

Q6. The significant effect of aldosterone is to

A. Decrease the renal reabsorption of sodium
B. Decrease the reabsorption of chloride
C. Decrease the rate of tubular reabsorption of potassium
D. Increase the rate of tubular reabsorption of sodium

Q7. Which one of the following is the potent stimulator of aldosterone secretion?

A. Increased potassium concentration
B. Increased sodium concentration
C. Decreased potassium concentration
D. Increased ECF volume

Q8. Release of aldosterone is stimulated by

A. Renin
B. Insulin
C. Thyroxine
D. Angiotensin II

Q9. Catecholamine hormones are synthesized in the

A. Zona fasciculata of the adrenal cortex
B. Chromaffin cells of the adrenal medulla
C. Zona glomerulosa of adrenal cortex
D. Zona reticularis of adrenal cortex

Q10. Which of the following hormones is required for uterine muscle contraction during child-birth?

A. Estrogen
B. Progesterone
C. ADH
D. Oxytocin

Q11. Action of antidiuretic hormone (ADH) leads to

A. Reabsorption of potassium from renal tubules
B. Excretion of hypotonic urine
C. Absorption of water from renal tubules
D. Decrease specific gravity urine

Q12. An increase in the osmolality of the extracellular compartment will lead to

A. Stimulation of ADH secretion
B. Stimulation of oxytocin secretion
C. Inhibition of ADH secretion
D. Stimulation of secretion of thyroxine

Q13. For catecholamine biosynthesis the rate-limiting enzyme is

A. Monoamine oxidase
B. DOPA decarboxylase
C. Phenylalanine hydroxylase
D. Tyrosine hydroxylase

Q14. Which of the following hormones cannot cross the blood–brain barrier?

A. Glucocorticoid
B. TSH
C. ACTH
D. Epinephrine

Q15. Pheochromocytomas are tumors of the

A. Pancreatic alpha cells
B. Adrenal cortex
C. Adrenal medulla
D. Thyroid gland

Q16. A characteristic of pheochromocytoma is elevated urinary excretion of

A. Phenylalanine
B. VMA
C. Tyrosine (VMA)
D. Dopamine

Q17. Epinephrine stimulates glycogenolysis in

A. Liver
B. Muscle
C. A and B
D. Kidney

Q18. Intake of coffee may

A. Stimulate secretion of epinephrine
B. Decrease secretion of glucagon
C. Stimulate secretion of GH
D. Stimulate secretion of LH

Q19. Presence of a significant amount of 5-HIAA in urine indicates

A. Renal condition
B. Cirrhosis of liver
C. Hyperthyroidism
D. Tumors of the enterochromaffin cells of the small intestine

Q20. Action of the parathyroid hormone leads to

A. Absorption of calcium at the tubular level
B. Increase in urinary excretion of phosphates
C. Increase in absorption of phosphates
D. A and B

Q21. Action of calcitonin causes

A. Urinary excretion of sodium
B. Increase in urinary calcium
C. Urinary excretion of calcium and inorganic phosphorus
D. Increase in urine potassium

Q22. Which of the following is the characteristic feature of hyperparathyroidism?

A. Low serum phosphorus
B. High serum phosphorus
C. High serum calcium
D. A and C

Q23. A 38-year-old male worker presented with muscle weakness, particularly with his affected thighs. He had difficulties, particularly climbing a staircase. On examination, it was found out that he had specific truncal obesity, moon face, and his blood pressure was 185/115. His blood reports were as follows: Elevated morning serum cortisol, Serum sodium: 150 mEq/L (Normal: 133–148 mEq/L), and serum potassium: 3.2 mEq/L (Normal: 3.8–5.6 mEq/L). The diagnosis is

A. Addison's disease
B. Cushing's syndrome
C. Graves' disease
D. Diabetes insipidus

Q24. A 57-year-old man presented with nausea, vomiting, loss of weight, loss of appetite, abdominal pain, extreme fatigue, low blood pressure, salt craving, hypoglycemia, and the appearance of patches of dark skin. His blood reports were as follows: Serum sodium: 120 mEq/L (Normal:133–148 mEq/L) and serum potassium: 6.2 mEq/L (Normal: 3.8–5.6 mEq/L). The diagnosis is

A. Addison's disease
B. Cushing's syndrome
C. Hashimoto's disease
D. Diabetes insipidus

Answers

1. A **2.** A **3.** B **4.** A **5.** A **6.** D **7.** A **8.** D **9.** B **10.** D
11. C **12.** A **13.** D **14.** D **15.** C **16.** B **17.** C **18.** A **19.** D **20.** D
21. C **22.** D

23. B: Cushing's disease, due to moon face, elevated values of cortisol, serum sodium, and low serum potassium.

24. A: Addison's disease, due to the specific symptoms, low serum sodium and high serum potassium

GASTROESOPHAGEAL REFLUX DISEASE (GERD)

LAQ: What is gastroesophageal reflux disease (GER)? Describe the pathophysiology, symptoms and treatment of GERD.

Ans: Gastroesophageal reflux disease (GERD) occurs when stomach acid frequently flows back into the esophagus. This acid reflux irritates the lining of the esophagus. Many individuals can manage the discomfort of GERD with lifestyle changes and over-the-counter medications. However, a significant number of persons with GERD require medications or surgery to manage related symptoms.

Pathophysiology

GERD is a lower esophageal sphincter (LES) dysfunction. The main cause of gastroesophageal reflux is the functional defect of the anti-reflux barriers at the esophagogastric junction. Normal LES status facilitates the transition of food from the esophagus into the stomach and prevents the reflux of gastric contents back into the esophagus.

The severity of GERD increases progressively with reflux which is mainly observed in the postprandial period. Hiatal hernia is associated with decreased LES pressure, decreased acid clearance, increased reflux, and more severe esophagitis. Esophageal mucosal inflammation affects nerves and muscles that alter LES function and esophageal body motility. A vicious cycle of inflammation and impaired motility leads to a progression of GERD. Patients with GERD may develop endoscopically visible erosive esophagitis.

Symptoms of GERD: The following are common signs and symptoms of GERD:

1. A burning sensation in the chest (heartburn), usually after eating, which might be worse at night
2. Chest pain
3. Difficulty in swallowing food
4. Regurgitation of food or sour liquid
5. Sensation of a lump in the throat

Factors responsible to the development of gastroesophageal reflux disease (GERD): The following are the factors responsible for GERD:

1. Disturbed structural and physiologic anti-reflux mechanisms at the gastroesophageal junction
2. Repeated episodes of reflux of gastric acid and bile, pepsin, digestive enzymes, etc
3. Disturbed esophageal clearance mechanisms
4. Drugs and alcohol that affect esophageal, LES, and gastric motility
5. Other factors such as delayed gastric emptying, esophagitis, and genetics.

Diagnosis: Examination of the upper gastrointestinal tract by endoscopy. This involves the insertion of an endoscope, which is a light and flexible tube with a tiny camera at the end. The endoscope is introduced through the throat into the esophagus and stomach. Endoscopy is an examination of the esophagus, stomach, and the initial part of the small intestine of the patient.

Treatment: The first line of treatment is the use of lifestyle changes and antacids to control excessive acid production in the stomach.

Lifestyle changes: Reduction of weight, strict control of alcohol, spicy food, smoking, and stress management.

Medications: The medicines used to treat GERD suppress acid production. The use of proton pump inhibitors (PPI) is considered the most effective part of treatment, which could help in the healing of the lining of the esophagus.

LABORATORY TESTS TO DETERMINE GASTRIC FUNCTIONS

LAQ: Describe gastric function tests.

Ans: The gastric function tests are performed using the gastric contents of a patient. Following is the general test procedure:

Histamine is a powerful stimulant for the secretion of hydrochloric acid in the normal stomach. Gastric contents are collected after the injection of histamine. The gastric specimens are collected by using a Rehfuss or a Ryle's tube. The collected specimens are analyzed for the determination of the following constituents:

1. Free acid
2. Combined acids
3. Bile
4. Blood
5. Lactic acid and
6. Starch

Principles

The strength of the acids is found by titrating them against standard 0.1 N sodium hydroxide. The titrations are carried out in two stages.

1. **Determination of strength of free acid:** It is carried out by titrating against 0.1 N sodium hydroxide and by using Topfer's reagent as an indicator. The combined acids (organic acids like protein hydrochloride, acid phosphates, butyric acid, citric acid, etc.) do not dissociate in the presence of free acid (hydrochloric acid). Hence first, free acid is neutralized at about pH 3.5. Hence an indicator like Topfer's reagent is used, having a pH range of 2.9 to 4.0 (color change from red to yellow).
2. **Determination of combined acids:** Afterward, as the pH increases with the addition of sodium hydroxide, the combined acids dissociate to give hydrogen ions which are capable of reacting with the added OH ions. The endpoint of this titration is nearer to pH 8.5. Hence phenolphthalein is used as an indicator. Its pH range is 8.3 to 10, and its color changes from colorless to pink.

Clinical significance of determination of total, free acid, and combined acid:

Ans: Determination of total, free acid and combined acid gives diagnosis of hyperchlorhydria, hypochlorhydria, achlorhydria and achylia gastrica.

Hyperchlorhydria is the term used when the maximum free acid exceeds 50 mEq/L. After the initial fall from a high fasting acidity, there may be a fairly rapid rise to concentrations exceeding 100 mEq/L. Such findings are seen occasionally in gastric ulcers and are most characteristic of duodenal ulcers. Blood, together with hyperchlorhydria, is suggestive of gastric ulcer and may also be found occasionally in gastric carcinoma. In pyloric stenosis, the volume of residual and resting contents may be very high with the presence of starch. A small percentage of cases of cancer of the stomach are found to have hyperchlorhydria.

In hypochlorhydria, there is a significant decrease in free acid, i.e. >20 mEq/L. It is associated with persons belonging to older age groups.

Achlorhydria term is used when there is no secretion of hydrochloric acid. The enzyme pepsin may be present. Achlorhydria is associated with persons belonging to older age groups, tuberculosis of the stomach, Addison's disease, ulcerative colitis, later stage of malignancy, and in pernicious anemia.

When free acid and the enzyme both are absent, the term achylia gastrica is used. It is observed in the later stage of malignancy and also in tuberculosis of the stomach.

The following are qualitative gastric function tests and related clinical significance.

1. Occult blood in the gastric specimen is used in the diagnosis and treatment of ulcers and also in malignant growths of the stomach, duodenum, and small and large intestines.
2. **Bile:** Bile may be found occasionally but is not usually of particular significance.
3. **Lactic acid:** Food remaining in the stomach may lead to the formation of lactic acid due to the fermentation, by the action of microorganisms. This may be observed in achlorhydria associated with the retention of food.
4. **Mucus:** Normally, mucus is present in small amounts. It is increased in gastric carcinoma and gastritis.

Multiple Choice Questions

Q1. Following are constituents of gastric secretions *except*

A. Hydrochloric acid
B. Pepsinogen
C. Acetic acid
D. Hemopoietic factor

Q2. The gastric secretion is produced by

A. Psychic factors
B. Gastrin
C. Digestive products in the intestine
D. All of the above

Q3. Which of these are strong stimulants of gastric hydrochloric acid?

A. Insulin B. Histamine
C. Ethyl alcohol D. All of the above

Q4. Hyperchlorhydria occurs when maximum free acid exceeds

A. 50–60 mEq/L B. 5–10 mEq/L
C. 10–15 mEq/L D. 15–30 mEq/L

Q5. Achlorhydria means

A. Free acid is 5–10 mEq/L
B. Free acid is 15–30 mEq/L
C. No secretion of free acid
D. Free acid is 10–15 mEq/L

Q6. Lactic acid may be present in gastric contents in

A. Achlorhydria associated with retention of food
B. Gastric ulcers
C. Hypochlorhydria
D. A and C

Q7. Achlorhydria may be due to all of the following *except*

A. Older age group
B. Tuberculosis of the stomach
C. Addison's disease
D. Cushing's syndrome

Q8. In gastric contents, blood together with hyperchlorhydria is suggestive of

A. Pyloric stenosis B. Gastric ulcers
C. Hyperchlorhydria D. All of the above

Q9. Which of these are absent in gastric contents in achyliagastrica?

A. Lactic acid B. Free acid
C. Pepsin D. B and C

Q10. These are all gastrointestinal hormones *except*

A. Gastrin B. Secretin
C. Cholecystokinin D. Renin

Q11. Survival of *H. pylori* in the stomach is dependent on the activity of

A. Peroxidase
B. Urease
C. Superoxide dismutase
D. A and C

Q12. Which of these can be used to treat acid indigestion?

A. Magnesium hydroxide
B. Aluminium hydroxide
C. Calcium carbonate
D. All of the above

Answers

1. C **2.** D **3.** D **4.** A **5.** C **6.** A **7.** D **8.** B **9.** D **10.** D
11. B **12.** D

ADYNAMIC ILEUS AND ACUTE COLONIC PSEUDO-OBSTRUCTION

LAQ: Write a note on adynamic ileus and colonic pseudo-obstruction.

Ans: Paralytic ileus (or adynamic ileus) is a condition in which there is a functional motor paralysis of the digestive tract secondary to neuromuscular failure involving the myenteric (Auerbach's) and submucous (Meissner's) plexus. The intestine fails to transmit peristaltic waves, resulting in a functional obstruction, to the movement of food ingredients under the process of digestion. Intestinal bacteria act on accumulated incompletely digested foods, which leads to the accumulation of fluid and gases in the intestine. It is the small intestine that is predominantly affected, but the colon and stomach could also be involved. The resultant intestinal content stasis leads to associated distension, vomiting, a decrease in bowel sounds, and constipation. Adynamic means lacking strength or force. Ileus refers to the intolerance of oral intake of foods and fluids, due to inhibition of the gastrointestinal force without signs of mechanical obstruction.

Adynamic ileus and colonic pseudo-obstruction cause functional obstruction of intestinal transit, due to uncoordinated or attenuated intestinal muscle contractions. Mechanical obstruction is not present. Ileus usually arises from an exaggerated intestinal reaction to abdominal surgery and it is often exacerbated by various other conditions.

Colonic pseudo-obstruction is induced by many metabolic disorders, several drugs that inhibit intestinal motility, severe illnesses, and extensive surgery. It presents massive colonic dilatation with variable and moderate small bowel dilatation.

First-line of treatment: Adynamic ileus and acute colonic pseudo-obstruction are initially treated with supportive measures such as correction of electrolyte abnormalities, intravenous rehydration, discontinuation of anti-kinetic drugs, and treatment of other contributing disorders. Neostigmine (an anticholinesterase drug) may be considered for the reversal of neuromuscular blockade.

HIRSCHSPRUNG DISEASE (HSCR)

Q: Describe Hirschsprung disease (HSRS), pathophysiology, symptoms, and treatment.

Ans: Hirschsprung disease is a genetic disorder. This disorder is a neurocristopathy and is characterized by the absence of the enteric ganglia along a variable length of the intestine. So far, eight genes are involved in HSCR. Incidences of HSCR occur five times more frequently in males than in females. Children with Down syndrome have a higher risk of having the disease.

In the last decades, the development of surgical approaches has dramatically decreased mortality and morbidity related to this genetic disorder with an incidence of 1/5000 live births.

Pathophysiology

In most cases, the diagnosis of HSCR is made in the newborn with an indication of intestinal obstruction with the following features:

1. Failure to pass early stool (meconium) within the first 48 hours of life
2. Abdominal distension that is relieved by rectal stimulation or enemas
3. Vomiting
4. Neonatal enterocolitis.

Some patients are diagnosed later in infancy or adulthood with severe constipation, chronic abdominal distension, vomiting, and failure to thrive.

Diagnosis: Examination of the patient by a physician and getting a medical history. The following tests are required to evaluate Hirschsprung's disease:

1. **Abdominal X-ray:** This radiological test may show a lack of stool in the large intestine or near the anus and dilated segments of the large and small intestine.
2. **Barium enema:** This procedure is performed to examine the large intestine for abnormalities. A barium meal is introduced into the rectum as an enema. An X-ray of the abdomen shows strictures, obstructions, and dilated intestine above the obstruction.
3. **Barium meal abdominal X-ray:** The findings of this test indicate a distended small bowel and proximal colon with an empty rectum. The classical image is a dilated proximal colon with the aganglionic cone narrowing towards the distal gut.
4. **Rectal biopsy:** In rectal biopsy tiny pieces of tissue from the rectal area are removed and examined by histopathology techniques to see if there are nerve cells in the rectum.
5. **Anorectal manometry:** This test measures nerve reflexes that are missing in Hirschsprung's disease.

Treatment: Specific treatment for Hirschsprung's disease is determined by a physician, based on

1. The extent of the clinical condition and
2. Child's age, overall health, and medical history

The treatment of HSCR is surgical. After careful preoperative management, the principle is to place the normal bowel at the anus and to release the tonic contraction of the internal anal sphincter. The surgeon removes the portion of the rectum and intestine that lacks normal nerve cells. The remaining portion is then connected to the anal opening. This is known as a pull-through procedure.

Chapter

18

Immunology

SAQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

Case Studies: Marks: 4 or 5

INTRODUCTION

Immunology is a branch of biology and medicine that covers the study of all aspects of the immune system. It deals with the physiological functioning of the immune system in states of both health and disease. Immunology considers malfunctions of the immune system in immunological disorders such as hypersensitivity, autoimmune diseases, immune deficiency, transplant rejection, and physical, chemical, and physiological characteristics of the components of the immune system.

The important primary lymphoid organs of the immune system include the thymus and bone marrow, and secondary lymphatic tissues such as the spleen, tonsils, lymph vessels, lymph nodes, adenoids, skin, and liver. Many components of the immune system are cellular and not associated with any specific organ but are embedded in the tissues or circulate in the blood.

Competency achievement: The student should be able to:

BI10.4: Describe and discuss innate and adoptive immune responses, self and nonself-recognition, and the central role of T-helper cells in immune responses

BAQ: Describe the following terms: Clinical immunology and serology.

Ans: Clinical Immunology is the study of diseases caused by disorders of the immune system. It also involves diseases of other systems, where immune reactions play a part in the pathology and clinical features.

The diseases caused by disorders of the immune system fall into two main categories:

1. Immunodeficiency, in which parts of the immune system fail to provide an adequate response, and
2. Autoimmunity, in which the immune system attacks its own host's body. Other immune system disorders include different hypersensitivities, such as allergies, in which the system responds inappropriately to harmless compounds. Clinical immunologists also deal with various ways to prevent transplant rejections.

Serology is the study of serum to diagnose infectious diseases by observing the immune antibody produced by the entry of the antigen (pathogen) into the body. It is the study of antigen–antibody or immunological reactions of the body by using a serum specimen.

IMMUNOLOGICAL REACTION AND RELATED TERMS

SAQ: Define immunity.

Ans: Immunity is the resistance to infection. It may be inherited or acquired from the mother.

SAQ: Define antigen and antibody.

Ans: An antigen is a substance or molecule when introduced into the body, triggers the production of an antibody by the immune system.

An antibody is a protein produced as a result of interaction with an antigen. It can combine with the specific antigen that stimulates its production.

SAQ: What is innate immunity?

Ans: Innate immunity is pre-existing resistance, and it is not acquired through contact with foreign (nonself) entities such as an antigen. It is nonspecific and includes barriers of infectious agents such as skin, mucous membranes, phagocytic cells, complement system, and inflammatory mediators.

SAQ: What is adaptive Immunity?

Ans: Adaptive immunity occurs after exposure to an infectious antigen. It is specific and mediated by antibodies or lymphocytes. It can be active or passive.

SAQ: What is immunization (vaccination)?

Ans: Immunization (vaccination) is an artificial infection given to an individual by introducing a non-virulent pathogen. The desired antibody is produced by the body to combat future infection.

SAQ: What is active immunity?

Ans: Active immunity is induced after contact with foreign antigens. This contact may be clinical or subclinical infection, immunization with live or killed infectious organisms or their antigens, exposure to microbial products (toxins), or transplantation of foreign cells. In all these instances, antibodies are produced by the host to combat the antigens, and lymphocytes acquire the ability to respond to the antigens.

SAQ: What is the advantage and disadvantage of active immunity?

Ans: The advantage of active immunity is the ability to acquire long-term protection against a specific antigen.

The disadvantage of active immunity is the slow onset of protection and the need for repeated or prolonged contact with the antigen.

SAQ: What is passive immunity?

Ans: In passive immunity, the antibody is produced outside the body of an individual in experimental animals such as rabbits or horses, etc. These antibodies are then collected from the animal sera and then given to an individual. This gives short-term protection from possible infection.

SAQ: What is the advantage and disadvantage of passive immunity?

Ans: The main advantage of passive immunity is the prompt availability of large amounts of antibodies.

The disadvantage of passive immunity is the short lifespan of the antibodies and sometimes the risk of hypersensitivity reactions if antibodies from another species are administered.

SAQ: What is chemotaxis?

Ans: Chemotaxis is a process through which phagocytic cells are attracted to the vicinity of invading pathogens.

SAQ: What is an epitope?

Ans: An epitope is a site on an antigen recognized by an antibody (Fig. 18.4).

SAQ: What are monoclonal antibodies?

Ans: Monoclonal antibodies are derived from one type of immunoglobulin-producing cell.

They have the same pattern of heavy and light chains, and they react specifically with the same antigen.

SAQ: What is a complement system?

Ans: Complement is a type of system containing 20 or more plasma proteins present in the globulin fraction of normal serum. It takes part in the primary immune reaction through various pathways and assists in rendering the pathogen inactive.

SAQ: Why in routine serological tests, the complement is inactivated?

Ans: In routine serological tests, complement is inactivated by heating the serum at 56°C for 20 minutes, so that it should not interfere in serological testing.

SAQ: Define titer.

Ans: Titer is the semi-quantitative measure of the amount of antibodies present in the serum.

SAQ: What are immunogens?

Ans: Immunogens are molecules that induce an immune response. In most cases, antigens are immunogens.

SAQ: What is a hapten?

Ans: A hapten is a molecule that is not immunogenic by itself but can react with a specific antibody. Haptens are usually small, but some high-molecular-weight nucleic acids are haptens. They are not immunogenic, because they cannot activate helper T cells. But they can stimulate a primary or secondary response when they bind to a carrier protein. The carrier protein activates helper T cells, and the hapten interacts with B cells bearing IgM specific for the hapten. The activated T cells stimulate the B cells to produce antibodies to the hapten.

SAQ: What are chemokines?

Ans: Chemokines are low molecular-weight proteins that stimulate leukocyte movement.

SAQ: What is the meaning of histocompatible?

Ans: Histocompatible means sharing major histocompatibility complex (transplantation) antigen.

SAQ: What is cell-mediated immunity?

Ans: Cell-mediated immunity is an immune response that involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.

SAQ: What is humoral immunity?

Ans: Humoral immunity means immunity in body fluid and is used to denote immunity mediated by complements and antibodies.

SAQ: What are cytokines? Give examples.

Ans: Cytokines are small cell-signaling protein molecules that are secreted by the glial cells of the nervous system and by numerous cells of the immune system. These are signaling molecules used extensively in intercellular communication. Examples: Interferon, erythropoietin, interleukins, etc.

Q: What is opsonin?

Ans: Opsonin is a substance capable of enhancing phagocytosis.

SAQ: What is macrophage and plasma cell?

Ans: Macrophage is a phagocytic mononuclear cell derived from bone marrow monocytes. It is found in tissues and at the site of inflammation.

A plasma cell is a terminally differentiated B cell that secretes antibodies.

SAQ: What are natural killer cells (NK cells)?

Ans: Natural killer cells are large granular lymphoid cells with no known antigen-specific receptors. They can recognize and kill certain virally infected cells. NK cells can activate the innate response.

Q: What is a major histocompatibility complex (MHC)?

Ans: The major histocompatibility complex (MHC) is a large genomic region found in most vertebrates that encodes MHC molecules. MHC molecules play an important role in the immune system and autoimmunity.

Q: What is T cell (T lymphocyte) and B cells?

Ans: T cell is a thymus-derived cell that participates in a variety of cell-mediated immune reactions.

B cells are lymphocytes that develop in the bone marrow in mammals. B cells are precursors of plasma cells that produce antibodies.

BAQ: Write a short note on the cluster of differentiation (CD).

Ans: The cluster of differentiation (CD) is a protocol used for the identification and investigation of cell surface molecules present in white blood cells. CD molecules can act in numerous ways, often acting as receptors or ligands important to the cell. A signal cascade is usually initiated, altering the behavior of the cell. Some CD proteins do not play a role in cell signaling but have other functions, such as cell adhesion. CD for humans is numbered up to 375, most recently (2016).

THE BASIC MECHANISMS OF INNATE IMMUNITY

BAQ: Write a note on the basic mechanisms of innate immunity.

Ans: Unlike the cells of the liver, heart, or lungs, the cells of the immune system are scattered throughout the body. They are present in the spleen, lymph nodes, bone marrow, and thymus and circulate through the blood and lymphatic fluid. These cells destroy any agent which they do not recognize as part of the body. These reactions of the immune system are activated if the first line of defense fails.

The first line of defense is provided by the intact skin and mucous membranes of the body. Due to the outer horny layer, the skin is the most resistant barrier, and the damp surface of the respiratory tract acts as a trapping mechanism with the action of hair-like cilia. The sebaceous secretions and sweat of the skin contain bactericidal and fungicidal fatty acids. Nasal secretions and saliva contain mucopolysaccharides capable of inactivating some viruses. Tears contain lysozyme, which is active against Gram-positive bacteria. Any microorganisms still present are destroyed by acid present in the stomach.

When the first line of defense has been overcome, the second line of defense comes into action. This includes the following actions:

1. The phagocytic activity of polymorphonuclear leukocytes of the blood and macrophages of reticuloendothelial cells. Microorganisms entering blood or tissue fluid are engulfed by these phagocytic cells.
2. The complement system, which leads to a series of complex events that involve various proteins (at least 9) to destroy bacterial cells.
3. Humoral immunity: It involves that part of the immune mechanism, which is meant to destroy the noncellular (humoral) antigens.
4. Cell-mediated immunity is designed to reject or destroy cellular antigens (cellular nonself).

ORIGIN OF IMMUNE CELLS

BAQ: Write a note on the origin of immune cells.

Ans: Stem cells in the bone marrow are the progenitors of all immune cells. Cytokines play an important role in the development of immune cells, such as polymorphonuclear cells, mast cells, monocytes, macrophages, and lymphocytes. Stem cells constantly divide and differentiate into various types of immune cells under the influence of cytokines. The bone marrow is ultimately responsible for the synthesis of eight types of cells: Red blood cells, platelets, neutrophils, basophils, eosinophils, mast cells, monocytes (macrophages), T lymphocytes and B lymphocytes. Some of these cell types mature in the bone marrow, while others migrate through the circulatory system and undergo final maturation in other tissues.

During embryonic development, blood cell precursors originate mainly in the fetal liver and yolk sac. In postnatal life, the stem cells reside in the bone marrow. Stem cells differentiate into cells of the erythroid, myeloid, or lymphoid series.

Lymphoid series evolve into two main lymphocyte populations: T cells and B cells. The ratio of T cells to B cells is approximately 3:1.

Stem cells lack T cell receptors and CD4 and CD8 molecules on their surface. During passage through the thymus, they differentiate into T cells that can express glycoproteins representing molecules such as CD3, CD4, CD8, etc. Within the thymus following two "thymic education" processes occur:

1. CD4-positive and CD8-positive cells bearing T cell receptors for self-proteins are killed by a process of programmed cell death called apoptosis. This results in self-tolerance to our proteins. This prevents autoimmune reactions.
2. CD4 and CD8 positive cells that do not react with self major histocompatibility complex (MHC) proteins are also killed.

These two processes are required for an effective immune response by T cells. These processes produce cells that are selected for their ability to react both with foreign antigens through their T cell receptors and with self-MHC proteins.

The earliest thymocytes express neither CD4 nor CD8 and are therefore classed as double-negative ($CD4^-CD8^-$) cells. As they progress through their development, they become double-positive thymocytes ($CD4^+CD8^+$) and finally mature to single-positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes that are then released from the thymus to peripheral tissues.

About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cells.

B cells do not pass through the thymus. Precursors of B cells mature in "gut-associated lymphoid tissue". Gut-associated lymphoid tissue is the mammalian equivalent of the bursa of Fabricius in birds. B cells are named for bursa and T cells for the thymus.

T CELLS

BAQ: Write a note on T-cells (T-lymphocytes).

Ans: T cells or T lymphocytes belong to a group of white blood cells known as lymphocytes. These cells play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells (NK cells), by the presence of a special receptor on their cell surface called T cell receptors (TCR). These cells are called T cells since the thymus is the principal organ responsible for the maturation of T cells. Several different subsets of T cells have been discovered, each with a distinct function. The following are the various types of T cells: Helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, and gamma-delta T cells.

BAQ: Write a note on Helper T Cells (T_H cells).

Ans: Helper T Cells (T_H cells) assist other white blood cells in immunologic processes. The main functions include the maturation of B cells into plasma cells and the activation of cytotoxic T cells and macrophages. These cells are also known as $CD4^+$ T cells because they express the CD4 protein on their surface.

Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of antigen presenting cells

(APCs). Once activated, they divide rapidly and secrete cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including T_{H1}, T_{H2}, T_{H3}, T_{H17}, or T_{FH}, which secrete different cytokines to facilitate a different type of immune response.

SAQ: Write one function for each of the cytotoxic T cells and helper T cells.

Ans: Cytotoxic T cells play a central role in cell-mediated immunity.

The main functions of helper T cells are the role in the maturation of B cells into plasma cells and the activation of cytotoxic T cells and macrophages.

SAQ: Write a note on cytotoxic T cells (T_C cells, or CTLs).

Ans: Cytotoxic T cells (T_C cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as $CD8^+$ T cells since they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigens associated with MHC class I, which is present on the surface of nearly every cell of the body.

SAQ: Write a note on memory T cells.

Ans: These are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen and provide memory to the immune system against past infections. Memory T cells comprise two subtypes: Central memory T cells (TCM cells) and effector memory T cells (TEM cells). Memory cells may be either $CD4^+$ or $CD8^+$.

SAQ: Write a note on regulatory T cells (T_{reg} cells).

Ans: These were formerly known as suppressor T cells. These cells play an important role in the maintenance of immunological tolerance. Their major role is to close T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escape the process of negative selection in the thymus. The naturally occurring T_{reg} cells and the adaptive T_{reg} cells are the two major classes of $CD4^+$ regulatory T cells. Naturally occurring T_{reg} cells arise in the thymus, whereas the adaptive T_{reg} cells may originate during a normal immune response.

SAQ: Write a note on natural killer T cells (NKT cells).

Ans: Natural killer T cells are a special kind of lymphocyte that bridges the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigens presented by a molecule called CD1d. The expression of CD1d molecules controls the magnitude of the cell-mediated immune response to an acute viral infection. Once activated, NKT cells secrete cytokines and release cytolytic molecules and function like T_H and T_C cells. They are also able to recognize and eliminate some tumor cells.

B CELLS

BAQ: Write a note on B cells.

Ans: B cells are lymphocytes that develop in the bone marrow in mammals. These are precursors of plasma cells that produce antibodies. B cells perform the following two important functions:

1. They differentiate into plasma cells and produce antibodies.
2. They are antigen-presenting cells (APCs).

During, embryogenesis, B cell precursors are recognized first in the fetal liver. From there, they migrate to the bone marrow, which is their main location during adult life. They do not require a thymus for maturation. The maturation of B cells has the following two phases:

1. The antigen-independent phase consists of stem cells, pre-B cells, and antigen-dependent B cells and,

2. The antigen-dependent phase consists of the cells that arise after the interaction of antigen with the B cells, e.g. activated B cells and plasma cells.

B cells constitute about 30% of the recirculating pool of small lymphocytes. Their life span is short about a few days to a few weeks. Approximately 10^9 B cells are produced each day. Within lymph nodes, they are located in germinal centers. Within the spleen, they are found in the white pulp. They are also found in gut-associated lymphoid tissue (e.g. Peyer's patches).

Each immunologically responsive B cell bears a surface receptor either IgM or IgD, that can react with one antigen. An antigen interacts with a B lymphocyte that shows the best "fit" with its immunoglobulin surface receptor. After binding to the antigen, the B cell is stimulated to proliferate and form a clone of cells. These selected B cells soon become plasma cells and secrete antibodies specific to the antigen. Plasma cells synthesize the immunoglobulins with the same antigenic specificity. Plasma cells secrete thousands of antibody molecules per second for a few days and then die.

CD4 CELLS

BAQ: Write a note on CD4 (cluster of differentiation 4) and CD8 (cluster of differentiation 8).

Ans: CD4 (cluster of differentiation) is a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. CD4 is a co-receptor that assists the T cell receptor (TCR) in activating its T cell following an interaction with an antigen-presenting cell. CD4 amplifies the signal generated by the TCR by recruiting an enzyme, known as the tyrosine kinase lck, which is essential for activating many molecules involved in the signaling cascade of an activated T cell. CD4 also interacts directly with MHC class II molecules on the surface of the antigen-presenting cell using its extracellular domain.

CD8 (cluster of differentiation 8) is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but it is specific for the class I MHC protein. The CD8 co-receptor is predominantly expressed on the surface of cytotoxic T cells, but can also be found on natural killer cells and dendritic cells.

SAQ: Write a brief note on natural killer cells.

Ans: Natural killer cells are active without prior exposure to the virus. These are not enhanced by exposure and are not specific to any virus. They are specialized in killing virus infected cells and tumor cells by secreting cytotoxins (perforins).

Natural killer cells are lymphocytes with some T cell markers. They do not have to pass through the thymus to mature. They do not have immunologic memory and T-cell receptors. They also do not require MHC proteins for killing antigens.

BAQ: Write a note on cytokines.

Ans: Cytokines are small cell-signaling protein molecules that are secreted by the immune system and also by glial cells of the nervous system. These are a category of signaling molecules used extensively in intercellular communication. Cytokines can be classified as proteins, peptides, or glycoproteins. Examples of cytokines are as follows: Brain-derived neurotrophic factor (BDNF), chemokines (CKs), epidermal growth factor (EGF), erythropoietin (EPO), fibroblast growth factor (FGP), interferon-α (IFN-α), interferon-β (IFN-β), interferon-γ (IFN-γ), insulin-like growth factor, interleukins 1–18 (IL-1 to IL-18), nerve growth factor (NGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF-α, TGF-β), tumor necrosis factors (TNF-α, TNF-β), etc. Cytokines have been classed as lymphokines, interleukins, and chemokines, based on their specific function, cell of secretion, or target of action.

Cytokines are released by cells into the circulation or directly into tissue. The cytokines locate target immune cells and interact with receptors on the target immune cells by binding to them. The interaction triggers or stimulates specific responses by the target cells.

Overproduction or inappropriate production of certain cytokines by the body can result in severe diseases as seen in various autoimmune diseases and also in respiratory viral infections. Acute respiratory distress syndrome (ARDS) seen in severe COVID-19 was due to very high levels of interleukins. ARDS leads to lung complications. It has been also found that interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) are produced in excess in rheumatoid arthritis, where they are involved in inflammation and tissue destruction.

BAQ: Enumerate the cytokines that play an important role in intercell communication.

Ans: Examples of cytokines that play an important role in intercell communication are as follows: Brain-derived neurotrophic factor (BDNF), chemokines (CKs), epidermal growth factor (EGF), erythropoietin (EPO), fibroblast growth factor (FGP), interferon-α (IFN-α), interferon-β (IFN-β), interferon-γ (IFN-γ), Insulin-like growth factor, interleukins 1–18 (IL-1 to IL-18), nerve growth factor (NGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF-α, TGF-β), tumor necrosis factors (TNF-α, TNF-β), etc.

BAQ: Write a note on interleukins.

Ans: Interleukins are a group of cytokines first seen to be expressed by white blood cells (leukocytes). The function of the immune system depends in large part on interleukins. The majority of interleukins are synthesized by helper CD4$^+$ T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T cells, B cells, and hematopoietic cells.

The various functions of some important interleukins are as follows:

1. IL-1 is a protein produced mainly by macrophages. It activates a wide variety of target cells, such as T and B lymphocytes, neutrophils, epithelial cells, and fibroblasts. These cells are activated by IL-1 to grow, differentiate or synthesize specific products.
2. IL-2: It is a protein produced mainly by helper T cells that stimulate both helper and cytotoxic T cells to grow.
3. IL-3 is produced by activated T helper cells, mast cells, NK cells, and eosinophils. IL-3 plays an important role in the differentiation and proliferation of myeloid progenitor cells, e.g. erythrocytes, granulocytes
4. IL-4 and IL-5 are proteins produced by helper T cells and differentiation of B cells, respectively. IL–5 enhances the synthesis of IgA and stimulates the production and activation of eosinophils, which play an important host defense against many helminths (worms) and are increased in immediate hypersensitivity (allergic) reactions.
5. IL-6 is produced by helper T cells and stimulates B cells to differentiate. It also induces fever (endogenous pyrogenic effect).

BAQ: Write a note on interferons.

Ans: Interferons (IFNs) are proteins made and released by lymphocytes in response to the presence of pathogens such as viruses, bacteria, tumor cells, and parasites. They allow communication between cells to trigger the protective defenses of the immune system to get rid of pathogens or tumors. IFNs are divided into three IFN classes: Type I IFN, Type II IFN, and Type III IFN.

The following are the functions of IFNs:

1. With the death of an infected cell, viral particles are released that can infect nearby cells. The infected cell warns neighboring

cells of a viral presence by releasing interferon. In response to interferons, inhibited protein synthesis destroys both the virus and infected host cells.

2. Interferon is responsible to upregulate major histocompatibility complex molecules, MHC I and MHC II.
3. IFNs activate immune cells, such as natural killer cells and macrophages.
4. IFNs increase recognition of infection or tumor cells by upregulating antigen presentation to T lymphocytes, and they increase the ability of uninfected host cells to resist new infection by the virus.

SAQ: What is erythropoietin (EPO)?

Ans: EPO is a glycoprotein hormone that controls red blood cell production (erythropoiesis). It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. It is produced by the peritubular capillary endothelial cells in the kidney and liver. Erythropoietin plays an important role in the brain's response to neuronal injury, and it is also involved in the wound-healing process.

SAQ: What are chemokines?

Ans: Chemokines are a family of small cytokines, secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells. Chemokines are found in all vertebrates, some viruses, and some bacteria. These proteins exert their biological effects by interacting with G protein-linked transmembrane receptors called chemokine receptors, which are specifically found on the surfaces of their target cells.

SAQ: Write a brief note on brain-derived neurotrophic factor (BDNF).

Ans: BDNF is a protein that is encoded by the BDNF gene. It is a member of the "neurotrophin" family of growth factors. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helps to support the survival of existing neurons, and encourages the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain areas, which are vital to learning, memory, and higher thinking. BDNF itself is important for long-term memory.

SAQ: Write a brief note on platelet-derived growth factor (PDGF).

Ans: PDGF is one of the numerous growth factors, that regulate cell growth and division. It plays a significant role in blood vessel formation (angiogenesis) and the growth of blood vessels from already-existing blood vessel tissue. Uncontrolled angiogenesis is a characteristic of cancer.

PDGF plays a role in embryonic development, cell proliferation, cell migration, and angiogenesis. It is also linked to several diseases, such as atherosclerosis, fibrosis, and malignant diseases.

SAQ: Write a brief note on fibroblast growth factors (FGFs).

Ans: These are a family of growth factors involved in angiogenesis, wound healing, and embryonic development. FGFs are important in the processes of proliferation and differentiation of a wide variety of cells and tissues. They are multifunctional proteins with a wide variety of effects. FHFs are most commonly mitogens that stimulate mitosis. These also have regulatory, morphological, and endocrine effects.

FUNCTIONS OF THE IMMUNE SYSTEM

Competency achievement: The student should be able to:

BI10.3: Describe the cellular and humoral components of the immune system and describe the types and structure of antibodies

LAQ: Describe cell-mediated and antibody-mediated immune system functions.

Ans: The main function of the immune system is to prevent infections by microorganisms.

The protection is provided primarily by the cell-mediated and antibody-mediated immune systems.

Cell-mediated immunity: It is an immune response that involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cellular immunity protects the body by:

1. Activating antigen-specific cytotoxic T-lymphocytes. These can induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;
2. Activating macrophages and natural killer cells, enabling them to destroy intracellular pathogens; and
3. Stimulating cells secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in removing virus-infected cells. However, it also participates in defending against bacteria, fungi, protozoans, and cancer cells. It also plays a major role in transplant rejection.

Cell-mediated immunity process (Fig. 18.1)

- When a microorganism (antigen) enters the body, it is ingested by a macrophage.
- The (antigen) microorganism is broken down, and fragments of it appear on the surface of the macrophage in association with class II major histocompatibility complex (MHC).
- The antigen-class II protein complex interacts with antigen-specific receptors on the surface of a helper T lymphocyte.

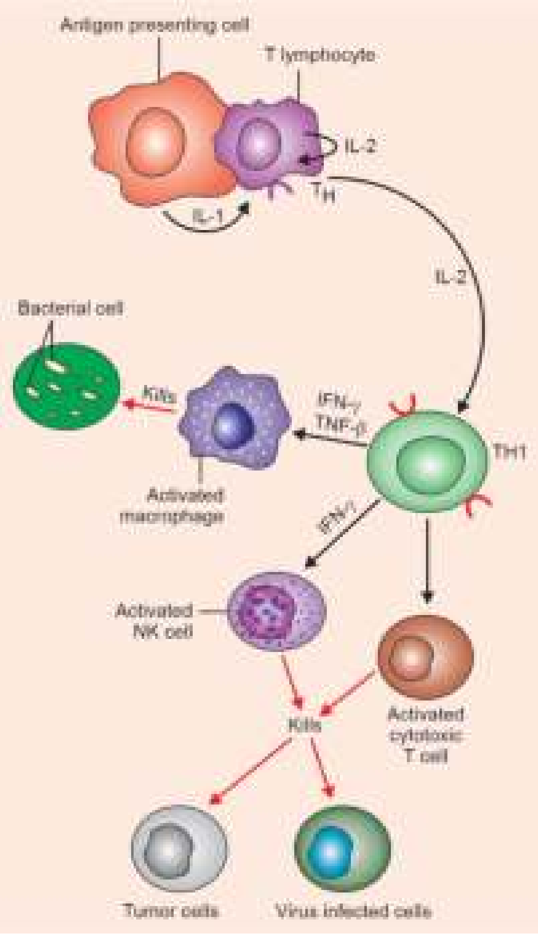


Fig. 18.1: Cell-mediated immunity process

- Activation and clonal proliferation of this antigen-specific helper T cell occur as a result of the production of interleukins.
- The most important interleukins produced are interleukin-1 (produced by macrophages) and interleukin-2 (produced by lymphocytes)
- These activated helper T cells mediate one important component of cellular immunity, i.e. delayed hypersensitive reaction.
- Cytotoxic T lymphocytes mediate another important component of the cellular immune response as follows:
  - When an antigen is inhaled and infects a cell, antigen glycoproteins appear

on the surface of the infected cell in association with class 1 MHC proteins. A cytotoxic T cell binds through its antigen-specific receptor to the viral antigen-class I protein complex and is stimulated to grow into a clone of cells. These cytotoxic T cells can specifically kill the infected cells by recognizing viral antigen-class I protein complexes on the cell surface.

Antigen-mediated immunity (humoral immunity response) (Fig. 18.2)

It is a humoral immune response that involves the production of antibodies to identify and neutralize foreign objects such as bacteria and viruses. The stages in this process are as follows:

1. Antigen detection
2. Activation of helper T cells
3. Antibody production by B cells

Antigen-mediated immunity process

- Antibody synthesis typically involves the cooperation of 3 cells: Macrophages, helper T cells, and B cells.
- After processing by a macrophage, fragments of antigen appear on the surface of the macrophage in association with Class II MHC proteins.
- These molecules bind to specific receptors on the surface of a helper T cell.
- Helper T cell produces lymphokines such as interleukin-2 (T cell growth factor), interleukin-4 (B cell growth factor)), and interleukin-5 (B cell differentiation factor). These factors activate the antigen-specific B cell.
- The activated B cell proliferates and differentiates to form many plasma cells.
- Plasma cells secrete a large amount of immunoglobulins (antibodies) (Fig. 18.2).
- Neutralizing antibodies are able for blocking the entry of a pathogen into a cell so that it fails to infect healthy cells.
- By Opsonization effect, antibodies bind to foreign particles and microorganisms, and make them more susceptible to the action of phagocytes.

Q: Give two examples each of active immunity and passive immunity.

Ans: Active immunity examples: Introduction of vaccines by intramuscular routes for the prevention of COVID-19, and Chickenpox infections.

Passive immunity examples: Hepatitis B gammaglobulin (IgG) preparation to protect against hepatitis B infection and varicella-zoster preparation to protect against varicella-zoster virus infection by intravenous administration.

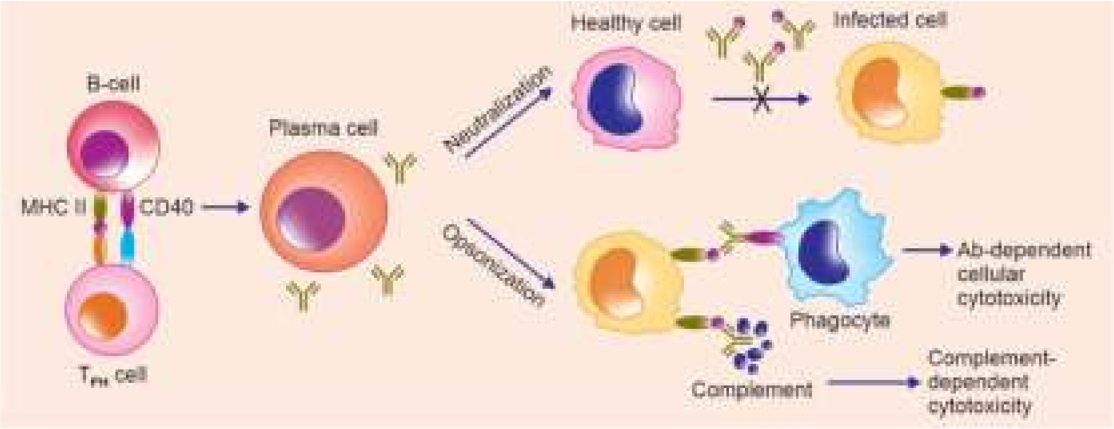


Fig. 18.2: Antigen-mediated (humoral) immune response

ANTIBODIES (IMMUNOGLOBULINS) (Fig. 18.3)

BAQ: What are antibodies?

Ans: Antibodies (immunoglobulins, Ig) are gamma globulin proteins that are found in blood or other bodily fluids. These are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. They are typically made of basic structural units with two large heavy chains and two small light chains to form, monomers (one unit), dimers (two units), or pentamers (five units). Antibodies are produced by plasma cells. Five different antibody isotypes are known in mammals, which perform different roles, and direct the appropriate immune response for each different type of foreign object they encounter. These antibodies are IgA, IgM, IgG, IgD, and IgE.

Q: What is a hypervariable region of antibodies?

Ans: Although the general structure of all antibodies is similar, a small region at the tip of the antibody-protein is extremely variable, allowing millions of antibodies with slightly different antigen binding sites. This region is known as the hypervariable region. Each of these variable sites can bind to a different antigen. The huge diversity of antibodies allows the immune system to recognize an equally wide variety of antigens.

LAQ: Write a note on antibodies.

Ans: Antibodies (immunoglobulins, Ig) are gamma globulin proteins that are found in blood or other bodily fluids. These are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. They are typically made of basic structural units with two large heavy chains and two small light chains to form, monomers (one unit), dimers (two units), or pentamers (five units) (Fig. 18.3).

Antibodies are produced by plasma cells. Five different antibody isotypes are known which perform different roles, and direct the appropriate immune response for each different type of foreign object they encounter.

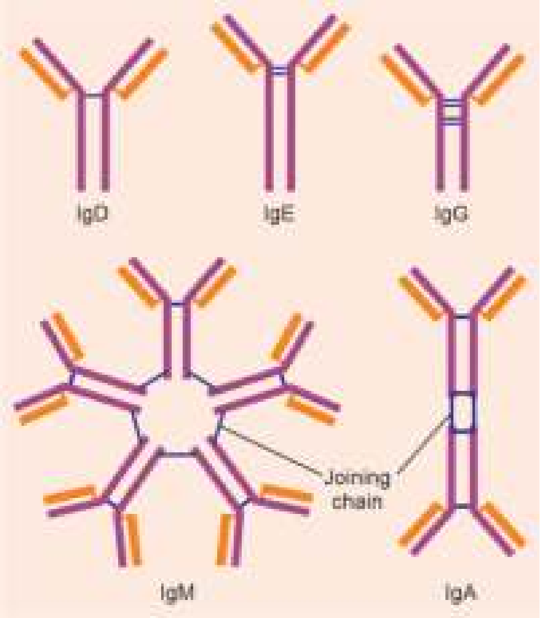


Fig.18.3: Various antibodies: Monomers (one unit): IgD, IgE, IgG; Dimer (two units): IgA and Pentamer: (five units) IgM

Antibodies are present in different varieties known as isotypes or classes. There are five antibody isotypes known as IgA, IgD, IgE, IgG, and IgM. They are each named with an "Ig" prefix that stands for immunoglobulin and another name for the specific antibody. These antibodies differ in their biological properties, functional locations, and ability to deal with different antigens.

Structure of an antibody: The main structure of an antibody is composed of heavy chains and light chains.

The heavy chain of antibodies: There are five types of mammalian Ig heavy chains denoted by the Greek letters: α, δ, ε, γ and μ. The type of heavy chain present defines the class of antibody, and these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and composition.

Each heavy chain has two regions, the constant region, and the variable region. The constant region is identical in all antibodies of the same isotype but differs in antibodies of different isotypes. The variable region of the heavy chain differs in antibodies produced by different B cells but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

Light chain: Lambda and kappa are two types of immunoglobulin light chains. A light chain has two successive domains: One constant domain and one variable domain. Each antibody contains two light chains that are always identical (Fig. 18.4).

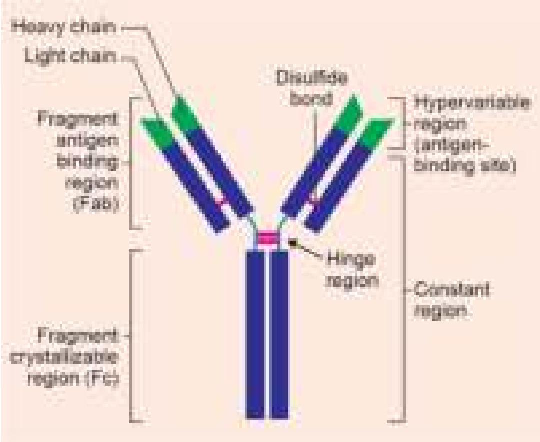

Heavy chain
Light chain
Fragment antigen binding region (Fab)
Disulfide bond
Hypervariable region (antigen-binding site)
Hinge region
Constant region
Fragment crystallizable region (Fc)

Fig. 18.4: Structure of an antibody

The unique and specific part of the antigen recognized by an antibody is called the epitope (Fig. 18.5). These epitopes bind with their antibody in a highly specific interaction, called induced fit, which allows antibodies to identify and bind only their unique antigen amid the millions of different molecules that make up an organism. The part of an antibody that binds to the epitope is called a paratope (Fig. 18.5). Recognition of an antigen by an antibody tag is meant for an attack by other parts of the immune system. Antibodies can also neutralize targets directly by binding to a part of a pathogen that may cause an infection.

Various Regions of Antibody

(Figs 18.4 and 18.5)

Some parts of an antibody have unique functions. The arms of the Y contain the sites that can bind two antigens and, therefore, recognize specific foreign objects. This region of the antibody is called the fragment antigen binding (Fab). It is composed of one constant and one variable domain from each heavy and light chain of the antibody.

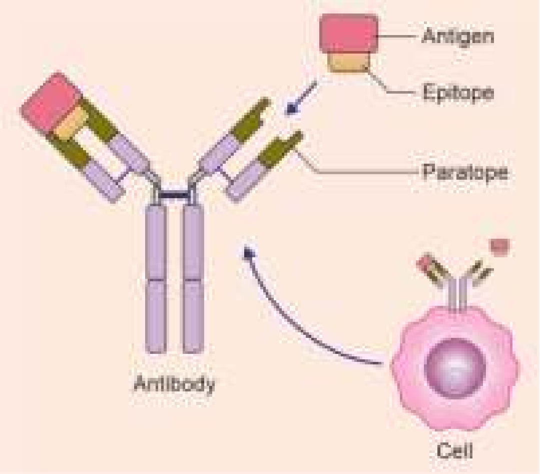


Fig. 18.5: Epitope and paratope regions

The base of the Y plays a role in modulating immune cell activity. This region is called the fragment crystallizable region (Fc). It is composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. Due to the Fc region, each antibody generates an appropriate immune response for a given antigen, by binding to a specific class of Fc receptors, and other immune molecules, such as complement proteins. Fc region mediates different physiological effects, including recognition of opsonized particles, lysis of cells, and degranulation of mast cells, basophils and eosinophils.

SAQ: What are natural antibodies?

Ans: Natural antibodies have been defined as antibodies that are produced without any previous infection, vaccination, other foreign antigen exposure, or passive immunization. Humans produce "natural antibodies," which are present in serum before viral infection. These antibodies can activate the classical complement pathway leading to the lysis of enveloped virus particles long before the adaptive immune response is activated.

SAQ: What is immunoglobulin diversity?

Ans: Virtually all microbes can trigger an antibody response. Successful recognition and eradication of many different types of microbes require diversity among antibodies. The amino acid composition of antibodies varies considerably and allows them to interact with many different antigens. It has been estimated that humans generate about 10 billion different antibodies, each capable of binding a distinct epitope of an antigen. Although a large number of different antibodies are generated in a single individual, the number of genes available to make these proteins is limited by the size of the human genome. Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes.

IMMUNOGLOBULIN CLASSES

Q: Draw and describe IgG (Fig.18.3).

Ans:

- Each IgG molecule consists of 2L chains and 2H chains (H2L2) linked by disulfide bonds.
- It is a divalent antibody, since it has 2 identical antigen-binding sites. Its molecular weight is approximately 1,50,000.
- There are 4 subclasses of IgG, based on antigenic differences in the H chains and the number and location of disulfide bonds. These subclasses are: IgG1, IgG2, IgG3 and IgG4.
- IgG is the predominant antibody in the secondary response and constitutes an important defense against bacteria and viruses.
- IgG is the only antibody to cross the placenta. It is, therefore, the most abundant immunoglobulin in newborns.
- It opsonizes bacteria, making them easier to phagocytose.

Q: Draw and describe IgA (Fig. 18.3).

Ans:

- IgA is the main immunoglobulin in secretions such as colostrum, saliva, tears, and secretions of tracts from respiratory, intestinal, and genital.
- It protects the mucous membrane from attack by bacteria and viruses.
- Molecular weight of each IgA molecule is approximately 400,000 and consists of 2H2L2 units plus one molecule each of the J (joining) chain and secretory component.
- The secretory component is a polypeptide synthesized by epithelial cells that provide for IgA passage to the mucosal surface. It also protects IgA from being degraded in the intestinal tract.

Q: Draw and describe IgM (Fig.18.3).

Ans:

- It is the main immunoglobulin produced early in the primary response. It is present as a monomer on the surface of virtually all B cells. There it functions as an antigen-binding receptor.
- In serum, it is a pentamer composed of 5H2L2 units plus one molecule of J (joining) chain.
- It is a pentamer, having a molecular weight of approximately 900,000. It has a total of 10 antigen-binding sites and a valence of 5–10.
- It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody reactions and plays an important role in defense against bacteria and viruses.
- It can be produced by the fetus in certain infections.

Q: Draw and describe IgD (Fig. 18.3).

Ans:

- Each IgD molecule consists of 2L chains and 2H chains (H2L2) linked by disulfide bonds.

- It is a monomeric antibody.
- It is present on the surface of many B lymphocytes. It is also present in small amounts in serum. It mainly functions as an antigen receptor.
- Molecular weight of IgD is approximately 1,84,000.

Q. Draw and describe IgE (Fig. 18.3).

Ans:

- Each IgE molecule consists of 2L chains and 2H chains (H2L2) linked by disulfide bonds.
- Molecular weight of IgE is approximately 190,000. It is present in trace amounts in normal serum (approximately 0.004%).
- The FC region of IgE binds to the surface of mast cells and basophils. Bound IgE serves as a receptor for antigen (allergen), and this antigen–antibody complex triggers allergic responses of immediate type (anaphylactic type) through the release of mediators. Persons with allergic reactivity have greatly increased the amount of IgE. IgE may appear in external secretions.
- Its concentration is typically increased during helminth (worm) infections.
- It does not fix complement and does not cross the placenta.

THE COMPLEMENT SYSTEM

BAQ: Write a note on the complement system.

Ans: The complement system is a biochemical cascade that helps the ability of antibodies to get rid of pathogens from an organism. It is a part of the innate immune system that is not adaptable and does not change throughout an individual's lifetime. However, it can be recruited and brought into action by the adaptive immune system.

The complement system consists of several small proteins (C1, C2, C3, etc.) found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. Over 25 proteins and protein fragments make up the complement system, including serum proteins, serosal proteins, and cell membrane receptors. They account for about 5% of the globulin fraction of blood serum.

The proteins and glycoproteins that constitute the complement system are synthesized by the liver hepatocytes. But significant amounts are also produced by tissue macrophages, blood monocytes, and epithelial cells of the genitourinary tract and gastrointestinal tract.

Three biochemical pathways activate the complement system:

1. The classical complement pathway,
2. The alternative complement pathway, and
3. The mannose-binding lectin pathway.

The following are the basic functions of the complement system:

1. Opsonization means enhancing phagocytosis of antigens
2. Chemotaxis means attracting macrophages and neutrophils
3. Lysis means rupturing membranes of foreign cells
4. Clumping of antigen-bearing agents
5. Altering the molecular structure of viruses

Multiple Choice Questions

Q1. The first line of defense is provided by

A. Phagocytic activity of polymorphonuclear leukocytes
B. Mucous membrane of the body
C. Intact skin
D. B and C

Q2. Stem cells differentiate into the following series belonging to

A. Lymphoid B. Myeloid
C. Erythroid D. All of the above

Q3. During embryonic development, blood cell precursors originate mainly in the

A. Fetal liver B. Yolk sac
C. Bone marrow D. A and B

Q4. Which of these cells play a central role in cell-mediated immunity?

A. Neutrophils
B. Eosinophils
C. Basophils
D. T lymphocytes

Q5. Which of these cells assist other cells in immunological processes?

A. Helper T cells B. Eosinophils
C. Basophils D. T lymphocytes

Q6. Which of these cells are capable of destroying virally infected cells and tumor cells?

A. Helper T cells
B. Eosinophils
C. Basophils
D. Cytotoxic T cells

Q7. Which of these cells play an important role in the maintenance of immunological tolerance?

A. Regulatory T cells
B. Helper T cells
C. Basophils
D. Eosinophils

Q8. B cells display surface

A. IgG B. IgM C. IgE D. IgA

Q9. HIV infection leads to a progressive reduction in

A. T cells with CD4 receptors
B. T cells with CD8 receptors
C. Basophils
D. Neutrophils

Q10. Cytokines have been classified as

A. Proteins B. Peptides
C. Glycoproteins D. All of the above

Q11. Interferons are released by

A. Neutrophils B. Eosinophils
C. Basophils D. Lymphocytes

Q12. Erythropoietin controls

A. White cell production
B. Red cell production
C. Platelet production
D. All of the above

Q13. Nitric oxide is one of the mediators made by

A. Neutrophils
B. Eosinophils
C. Macrophages
D. Lymphocytes

Q14. Which of this is the only antibody that crosses the placenta?

A. IgM B. IgD C. IgA D. IgG

Q15. Which of this is the main antibody produced early in the primary response?

A. IgM B. IgD C. IgA D. IgG

Q16. Concentration of which of this antibody increases typically in helminth infections?

A. IgM B. IgD C. IgE D. IgG

Q17. Which is the main immunoglobulin present in tears and saliva?

A. IgA B. IgG
C. Lysozyme D. None of the above
E. All of the above

Q18. Macrophages

A. Are produced by differentiation of monocytes
B. Produce nitric oxide
C. Are phagocytes
D. A, B, and C

Q19. Phagocytosis is

A. Carried out by cells of the adaptive immune system
B. Restricted to macrophages
C. Important in bacterial infections
D. All of the above

Q20. Both mast cells and basophils

A. Are phagocytic
B. Are found primarily in lymph nodes
C. Have receptors for IgM antibodies
D. Release histamine

Q21. Viral replication within cells is inhibited by

A. IL-4 B. IL-1
C. IFN-α D. TNF-α

Q22. Cytotoxic T cells generally recognize antigens in association with

A. Class I MHC determinants
B. Class II MHC determinants
C. Class III MHC determinants
D. HLA-DR determinants
E. All of the above

Q23. The antibody-binding site is formed primarily by

A. The constant regions of H and L chains
B. The variable regions of L chains.
C. The hypervariable regions of H and L chains
D. The hypervariable regions of H chains

Q24. Which one is the class of immunoglobulin present in the highest concentration in the blood of a newborn?

A. IgM B. IgG
C. IgA D. IgD

Q25. Antigen-presenting cells that activate helper T cells must express which one of the following on their surfaces?

A. IgE
B. IgM
C. Class II MHC antigens
D. Class I MHC antigens

Q26. Graft and tumor rejection are mediated primarily by

A. Noncomplement-fixing antibodies
B. Cytotoxic T cells
C. Helper T cells
D. B cells

Q27. Which one of the following statements best explains the relationship between inflammation of the heart (carditis) and infection with group A beta-hemolytic streptococci?

A. Streptococci are ingested by neutrophils that release proteases that damage heart tissue
B. Streptococcal antigens induce antibodies cross-reactive with heart tissue
C. Streptococcal antigens bind to IgE on the surface of heart tissue and histamine is released
D. A and C

Q28. The role of the macrophage during an antibody response is to

A. Processing and presentation of antigen
B. Lyse virus-infected target cells
C. Activate cytotoxic T cells
D. B and C

Q29. The main advantage of passive immunization over active immunization is that

A. It can be administered orally
B. Antibody persists for a longer period
C. It provides antibodies more rapidly
D. It contains primarily IgG

Q30. Each of the following statements concerning the variable regions of heavy chains and the variable regions of light chains in a given antibody molecule is correct *except*

A. They contain the hypervariable regions
B. They define the specificity of antigen
C. They are encoded on different chromosomes
D. They have the same amino acid sequence

Q31. Each of the following statements concerning class II MHC proteins is correct *except*

A. They are found on the surface of all nucleated cells
B. They have a high degree of polymorphism
C. They are involved in the presentation of antigens by macrophages
D. They have a binding site for CD4 proteins

Q32. Each of the following statements concerning a hybridoma cell is correct *except*

A. The antibody produced by a hybridoma cell is heterogeneous
B. The myeloma cell component provides the ability to grow indefinitely
C. Unfused myeloma cells cannot grow
D. The antibody produced by a hybridoma cell is homogeneous

Answers

1. D **2.** D **3.** D **4.** D **5.** A **6.** D **7.** A **8.** B **9.** A **10.** D
11. D **12.** B **13.** C **14.** D **15.** A **16.** C **17.** A **18.** D **19.** C **20.** D
21. D **22.** A **23.** C **24.** B **25.** C **26.** B **27.** B **28.** A **29.** C **30.** D
31. A **32.** A

Competency achievement: The student should be able to:

BI10.5: Describe antigens and concepts involved in vaccine development

Competency achievement: The student should be able to:

PE19.3: Vaccine description with regards to the classification of vaccines, strain used, dose, route, schedule, risks, benefits, side effects, indications, and contraindications

VACCINES

LAQ: Describe the terms vaccine and vaccination. Describe the classification of vaccines and various concepts and methods involved in vaccine development.

Ans: A vaccine is a biological preparation that provides active acquired immunity to a specific disease. A vaccine contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. The agent stimulates the immune system of the body to recognize the agent as a threat and then immune system mechanisms destroy it. The component in the vaccine facilitates the defense mechanism of the body to further recognize and destroy any of the microorganisms associated with it and also to encounter them in the future. Vaccines can be prophylactic or therapeutic. The administration of vaccines is called vaccination.

Classification of vaccines

The following are the main types of vaccines:

1. Live-attenuated vaccines,
2. Inactivated vaccines
3. Subunit vaccines
4. Toxoid vaccines
5. Conjugate vaccines
6. DNA vaccines and
7. Recombinant vector vaccines.

1. **Live attenuated vaccines:** An attenuated vaccine is created by reducing the virulence of a pathogen. It is kept viable or "live". Attenuation makes an infectious agent harmless or less virulent. Live attenuated vaccines are used to protect against measles, mumps, rubella, rotavirus, smallpox, chickenpox, yellow fever, etc.
2. **Inactivated vaccines:** Inactivated vaccines are made from the killed version of the microorganism that causes a disease. To produce this type of vaccine, bacteria or viruses are killed or inactivated by chemical treatment or heat. Inactivated vaccines usually do not provide immunity that is as strong as live vaccines. Hence patients may need several doses over time (booster shots) to get ongoing immunity against diseases. Examples of inactivated vaccines are: Inactivated poliovirus Covaxin (on COVID 19), inactivated polio vaccine (IPV), pertussis vaccine, rabies vaccine, and hepatitis A virus vaccine.
3. **Subunit vaccines:** Subunit vaccines use specific antigens of the microorganism, which may be protein, carbohydrate, or capsid of a microorganism. Since these vaccines use only specific antigens of the microorganism, they give a very strong immune response that is targeted to specific parts of the microorganism. One may need booster shots to get ongoing protection against specific diseases. These vaccines are used to protect against pneumococcal disease, *Haemophilus influenzae* type b disease, hepatitis B virus, human papillomavirus, whooping cough, meningococcal disease, shingles, etc.
4. **Toxoid vaccines:** Toxoid vaccines use a toxin made by the microorganism that causes a disease. They create immunity to the parts of the microorganism that cause disease instead of the microorganism itself. That means the immune response is targeted to the toxin instead of the whole organism. Toxoid vaccines are used to protect against diphtheria and tetanus.

5. **Conjugate vaccines:** Conjugate vaccines combine a strong antigen with a weak antigen so that the immune system has a stronger response to the weak antigen. The antigen of some pathogenic bacteria does not elicit a strong response from the immune system. Hence, vaccination against this weak antigen would not protect the person later in life. In this case, a conjugate vaccine is used to invoke an immune system response against the weak antigen.

   In a conjugate vaccine, the weak antigen mainly is a polysaccharide that is attached to a strong protein antigen. The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine. Other pathogens that are combined in a conjugate vaccine to increase an immune response are *Streptococcus pneumoniae* and *Neisseria meningitidis*. both of which are conjugated to protein carriers like those used in the Hib conjugate vaccine.

6. **DNA Vaccines:** DNA vaccines are produced using genetically engineered DNA of the microorganism.

   DNA vaccination is a technique for protecting against disease by injection with genetically engineered DNA along with a specific adjunct (additional component) so that genetically engineered DNA could reach the cells of the host and directly produce an antigen, generating a protective immunological response.

   This method of vaccine preparation offers a number of potential advantages over traditional approaches. These include the stimulation of both B- and T-cell responses, improved vaccine stability, the absence of any infectious agent, and the manufacturing of DNA vaccines on a large scale.

   DNA vaccines are prepared against COVID-19, influenza virus, hepatitis B virus, malarial parasites, human immunodeficiency virus, rabies virus, and mycoplasmas.

   The Pfizer/BioNTech vaccine and Moderna vaccines for COVID-19 are examples of DNA vaccines.

7. **Recombinant vector vaccines:** Recombinant vector vaccines are prepared using an attenuated virus or bacterium to introduce microbial DNA to cells of the body. Vector refers to the virus or bacterium used as the carrier. Usually, viruses adhere to specific cell receptors and inject their genetic material into them. Recombinant vector vaccines closely mimic a natural infection and therefore perform well by stimulating the immune system. Examples of recombinant vector vaccines are Covishield against COVID-19 and Gardasil vaccine against human papillomavirus (HPV).

USE OF HUMAN CELL STRAINS IN VACCINE DEVELOPMENT

Q: Explain the use of human cell strains in vaccine development.

Ans: Vaccines developed using human cell strains: The number of lives saved and morbidity reduction associated with the discovery of the first human cell strain used for the production of licensed human virus vaccines, known as WI-38. The diseases studied include measles, mumps, poliomyelitis, rubella, adenovirus, rabies varicella (chickenpox), herpes zoster, and hepatitis A.

Results indicate that the total number of cases of poliomyelitis, measles, mumps, rubella, varicella, adenovirus, rabies, and hepatitis averted or treated with WI-38-related vaccines was 198 million in the US and 4.5 billion globally. The total number of deaths averted from these same diseases was approximately 450,000 in the US and 10.3 million globally.

Human cell lines (strains) are used for the development of vaccines. Human cell lines (strains) are cells developed *in vitro* using a specific culture medium. It is necessary to handle all human cell lines carefully and must be handled as potential biohazards The WI-38 cell strain was developed in 1962 in the United States, and the MRC-5 cell strain (also started with fetal lung cells) was developed, using the Hayflick phenomenon, in 1970 at the Medical Research Center in the United Kingdom. The Hayflick phenomenon is the number of times a normal somatic human cell population will divide before cell division stops. However, this limit does not apply to stem cells.

WI-38 (Wister Institute-38) cell line is the first human diploid cell line used in human vaccine preparation. WI-38 cells were isolated from the lung tissue of a 3-month-old, female, embryo.

MRC-5 (Medical Research Council cell strain 5) is a diploid cell culture line composed of fibroblasts, originally developed from the lung tissue of a 14-week-old aborted Caucasian male fetus.

The following vaccines were developed using either the WI-38 or the MRC-5 cell strains:

1. Varicella (chickenpox) vaccine
2. Zoster (shingles) vaccine
3. Hepatitis A vaccines
4. Adenovirus Type 4 and Type 7 oral vaccine
5. Rabies vaccine
6. Rubella vaccine

SAQ: What is the difference between live vaccines and killed (inactivated) vaccines?

Ans: Live virus vaccines use the attenuated (weak) form of the virus. Killed (inactivated) vaccines are made from a protein or other small part taken from a virus or bacteria.

Live vaccines are not recommended for highly immunocompromised persons and during pregnancy.

Live vaccines can potentially cause an infection in highly immunocompromised persons including HIV patients. Similarly, the live attenuated virus in a vaccine could cross the placenta and result in viral infection of the fetus.

EPIDEMIOLOGY OF VACCINE-PREVENTABLE DISEASES

Competency achievement: The student should be able to:

PE19.2: Explain the epidemiology of vaccine-preventable diseases

LAQ: Describe the epidemiology of vaccine-preventable diseases.

Ans: Vaccine epidemiology is the study of the interactions and effects of vaccines and vaccination programs on the epidemiology of vaccine-preventable diseases.

The terms "vaccine" and "vaccinology" came into use after Edward Jenner discovered the smallpox vaccine. The word "vaccine" originated from *vacca*, a Latin term for the cow. The credit for the first use of the term "vaccine" goes to Swiss physician Louis Odier (1748–1817), and the terms "vaccination" and "to vaccinate" were first used by Richard Dunning (1710–1797).

Louis Pasteur (27 December 1822–28 September 1895) was a French chemist and microbiologist known for his discoveries of the principles of vaccination, microbial fermentation, and pasteurization.

The next routinely recommended vaccines were developed early in the 20th century. These include vaccines that protect against pertussis (1914), diphtheria (1926), and tetanus (1938). These three vaccines were combined in 1948 and given as the DTP vaccine. The polio vaccine was licensed in 1955.

Recommended vaccines in late 1950 were smallpox, diphtheria, tetanus, pertussis, and polio (IPV). These were given in combination as DTP.

In 1963, the measles vaccine was developed, and by the late 1960s, vaccines were also available to protect against mumps (1967) and rubella (1969). These three vaccines were combined into the MMR vaccine by Dr. Maurice Hilleman in 1971.

The vaccine for *Haemophilus influenzae* type b was licensed in 1985 and was recommended in 1989. When the schedule was published again in 1994, the hepatitis B vaccine had been added.

The following important changes were included to the schedule between 1995 and 2010: New vaccines: Varicella (chickenpox-1996), rotavirus (1998–1999; 2006, 2008); hepatitis A (2000); pneumococcal vaccine (2001), influenza (2002); hepatitis A (2006).

Since then, there have been the following different types of vaccines in use: Live, killed, conjugate, component, and recombinant vaccines. While live vaccines protect the administration of a single dose, non-live vaccines usually require multiple doses for a satisfactory primary response. A minimum of 4 weeks interval is required between successive doses. A longer interval (about 8 weeks) results in higher antibody levels. The booster doses are generally given 6 or more months after the completion of the primary series. The booster doses have rapid and higher antibody responses,

The following various vaccines are currently in use: Chickenpox vaccine, DTaP immunization (vaccine), Hib vaccine, HPV vaccine, hepatitis A vaccine, hepatitis B vaccine, pneumococcal conjugate vaccine, pneumococcal polysaccharide vaccine, polio immunization (vaccine), influenza vaccine, meningococcal vaccine, shingles vaccine, Tdap vaccine, MMR vaccine, rotavirus vaccine, tetanus vaccine.

Principles of epidemiology include:

1. Understanding the pattern of disease by geographical, rural–urban, and gender variations,
2. The linkage between disease burden and immunization coverage.

The specific points considered are:

1. Which time of the year the specific mass immunization campaign should be conducted?
2. For conducting mass campaigns, which age group should be targeted?
3. Where should immunization efforts be concerted?
4. Why do outbreaks of specific diseases occur?
5. Why some children do not suffer from the disease even though they have not received any vaccination?

The learning and study of vaccine epidemiology is useful to:

1. Study and take decisions on how to choose vaccines to include in a public health program;
2. Assess the burden of the disease;
3. Identify target pathogens for vaccine research;
4. Identify sources and transmission pathways of disease-causing microorganisms;
5. Determine vaccination strategies;
6. Design disease-specific control, elimination, and eradication strategies;
7. Monitor performance indicators of the vaccine;
8. Take steps to improve surveillance; and
9. Measure the progress and impact of vaccination strategies.

COMPONENTS OF THE UNIVERSAL IMMUNIZATION PROGRAM AND THE SUBNATIONAL IMMUNIZATION PROGRAM

Competency achievement: The student should be able to:

PE19.1: Explain the components of the universal immunization program and the subnational immunization program

BAQ: Explain the components of the universal immunization program (UIP) and the subnational immunization program (SIP).

Ans: The following are the important features of UIP:

1. Under UIP, immunization is provided free of cost against 12 vaccine-preventable diseases:

A. Nationally free of cost treatment against the following 9 diseases: Diphtheria, pertussis, tetanus, polio, measles, rubella, severe form of childhood tuberculosis, hepatitis B and meningitis and pneumonia caused by *hemophilus influenza* type B

B. Sub-nationally free-of-cost treatment against the following 3 diseases: Rotavirus diarrhea, pneumococcal pneumonia, and Japanese encephalitis

2. A child is said to be fully immunized if the child receives all due vaccines as per the national immunization schedule within 1st year age of the child.
3. The two major milestones of UIP have been the elimination of polio in 2014 and maternal and neonatal tetanus elimination in 2015.

BAQ: Write a note on side effects and safety matters related to vaccines.

Ans: The vaccines meant to prevent diseases in children are very important since the diseases that childhood vaccines are meant to prevent are most likely to occur when a child is very young and the risk of complications is greatest. Hence, early vaccination in the case of children is essential. However, any vaccine can cause side effects. Usually, these side effects are minor. The following are the commonly observed side effects: A low-grade fever, fussiness, and soreness at the injection site. Some vaccines cause temporary headaches, fatigue, or loss of appetite. Rarely, a child may experience a severe allergic reaction or a neurological side effect, such as a seizure. Although these rare side effects are a concern, the risk of a vaccine causing serious harm or death is extremely small. The benefits of getting a vaccine are much greater than the possible side effects for almost all children.

SAQ: What is the most important precaution taken in the case of vaccination?

Ans: Vaccines are not given to children or any adult who has known allergies to specific vaccine components. If a child or adult individual develops a life-threatening reaction to a particular vaccine, further doses of that vaccine will not be given.

SAQ: Enumerate names of common vaccines.

Ans: Chickenpox vaccine, DTaP immunization (vaccine), Hib vaccine, HPV vaccine, hepatitis A vaccine, hepatitis B vaccine, pneumococcal conjugate vaccine, pneumococcal polysaccharide vaccine, polio immunization (vaccine), influenza vaccine, meningococcal vaccine, shingles vaccine, tdap vaccine, MMR vaccine, rotavirus vaccine, tetanus vaccine.

VACCINATION OF CHILDREN

BAQ: Give an account of vaccines given to a child at birth.

Ans: Vaccination at birth

Bacillus Calmette-Guérin (BCG): A single dose vaccine. This vaccine offers protection against tuberculosis. Administered via injection on the upper arm.

Potential side effects: Soreness or discharge at the site of the injection, fever, headache, swelling of lymph nodes under the armpit on the arm that receives the vaccine.

Oral polio vaccine (OPV)-0: This vaccine protects against the poliovirus.

This is the first dose taken at birth. The next dose is taken when the child is 6 weeks old, the third dose at 10 weeks old, and the last dose at 14 weeks old. OVP is administered orally.

Potential side effects: No specific common side effects are associated with this vaccine.

Hepatitis B at birth dose. This is a single-dose vaccine. This vaccine protects against hepatitis B virus. Administered via injection Potential side effects: Some redness and soreness at the site of the injection.

BAQ: Give an account of vaccines given to a child 6 weeks after birth.

Ans: Vaccination: 6 weeks after birth

Oral polio vaccine (OPV)-1: This is the second OPV dose taken at 6 weeks. The next dose is taken when the child is 10 weeks old, and the last dose at 14 weeks old. Administered orally.

Potential side effects: There are no significant side effects.

Pentavalent-1: This vaccine offers protection against diphtheria, pertussis, tetanus, hepatitis B and Hib.

This is the first dose taken at 6 weeks old. The next dose is taken when the child is 10 weeks old and the last dose at 14 weeks old. Administered via injection.

Potential side effects: Fever for 1–3 days, pain, redness, and swelling may occur at the site of the injection.

Rotavirus vaccine (RVV)-1: This vaccine offers protection against rotaviruses which are the most common cause of severe diarrhea in infants and young children.

This is the first dose. The second dose is taken when the child is 10 weeks old and the last dose at 14 weeks old. Administered orally.

Potential side effects: Mild irritation, diarrhea, and vomiting.

Pneumococcal conjugate vaccine (PCV)-1: This vaccine offers protection against pneumococci that may cause pneumonia, meningitis, and infections such as sinusitis and otitis media in children.

This is the first of two doses of the PCV. The second dose is taken when the child is 14 weeks old. Administered via injection.

Potential side effects: Fever, chills, irritability. headache, pain, redness, and swelling at the site of injection.

If OVP is not preferred, then following vaccine could be used.

Inactivated polio vaccine (IPV)-1: This vaccine offers protection from the poliovirus. This is the first of two doses of the IPV. The second dose is given to child at 14 weeks. Administered via injection.

Potential side effects of this vaccine include fever and soreness.

BAQ: Give an account of vaccines given to a child 10 weeks after birth.

Ans: Vaccination: 10 weeks after birth

Pentavalent-2: This vaccine offers protection against diphtheria, pertussis, tetanus, hepatitis B and Hib.

The second dose is taken when the child is 10 weeks old and the last dose at 14 weeks old. Administered via injection.

Potential side effects: Fever for 1–3 days, pain, redness, and swelling may occur at the site of the injection.

Oral polio vaccine (OPV)-2: This vaccine protects against the poliovirus.

This is the third OPV dose taken when the child is 10 weeks old. The last dose is taken when the child is 14 weeks old. Administered orally.

Potential side effects: There are no common side effects.

Rotavirus vaccine (RVV)-2: The second dose is taken when child is 10 weeks old and the last dose at 14 weeks old. This vaccine offers protection against rotaviruses. Administered via injection. Administered orally.

Potential side effects: Mild irritation, diarrhea, and vomiting.

BAQ: Give an account of vaccines given to a child 14 weeks after birth.

Ans:

Vaccination: 14 weeks after birth

Pentavalent-3. This vaccine offers protection against diphtheria, pertussis, tetanus, Hepatitis B and Hib.

This is the last pentavalent vaccine dose to be taken at 14 weeks old. Administered via injection.

Potential side effects: Fever for 1–3 days, pain, redness, and swelling may occur at the site of the injection.

Oral polio vaccine (OPV)-3: This is the last OPV dose taken when the child is 14 weeks old. This vaccine protects against the poliovirus. Administered orally.

Potential side effects: There are no common side effects.

Rotavirus vaccine (RVV)-3: This vaccine offers protection against rotaviruses.

This is the last RVV dose taken when the child is 14 weeks old. Administered via injection. Administered orally.

Potential side effects: Mild irritation, diarrhea, and vomiting.

Pneumococcal conjugate vaccine (PCV) -2: This vaccine offers protection against Pneumococci that may cause, pneumonia, meningitis, and infections such as sinusitis and otitis media in children.

The second of two doses of the PCV is given at 14 weeks old. Administered via injection.

Potential side effects: Fever, chills, irritability, headache, pain, redness, and swelling at the site of injection.

If OVP is not preferred, then following vaccine could be used:

Inactivated polio vaccine (IPV)-2: This vaccine offers protection from the poliovirus The final IPV dose is given to the child at 14 weeks. The second dose is given to child at 14 weeks. Administered via injection. Potential side effects of this vaccine include fever and soreness.

BAQ: Give an account of vaccines given to a child 9–12 months after birth.

Ans: Vaccination: 9–12 months after birth

Measles and rubella (MR)-1

The first of two doses of the MR vaccine. The second dose is administered to a child between 16 and 24 months old. This vaccine offers protection against measles and rubella. Administered via injection.

Potential side effects of this vaccine include: Swelling, redness, and sore feeling for 2 to 3 days.

High temperature for 2–3 days. After around 7 to 11 days after the injection, the child may feel unwell.

Note

Japanese encephalitis (JE) vaccine is advised for families who plan to visit or live in a country where JE occurs.

Japanese encephalitis (JE-1): This vaccine offers protection against Japanese encephalitis. This is the first of two doses of the JE-1 vaccine. The second dose is given to the child between 16 and 24 months. Administered via injection.

Potential side effects: Fever, headache, muscle pain, tenderness, redness, or swelling around the vaccine shot.

Pneumococcal conjugate vaccine: Booster*. This vaccine offers protection against pneumococci that may cause, pneumonia, meningitis, and infections such as sinusitis and otitis media in children. This is a single-dose vaccine. Administered via injection.

Potential side effects: Loss of appetite, irritability, redness, and swelling at the site of injection.

BAQ: Give an account of vaccines given to a child 16–24 months after birth.

Ans: Vaccination: 16–24 months after birth

Measles and rubella (MR)-2. The vaccine offers protection against measles and rubella.

The second of two doses of the MR vaccine is to be taken by the child between 16 and 24 months. Administered via injection.

Potential side effects: High fever for 2–3 days, redness, and swelling at the site of the injection.

The final Japanese encephalitis (JE) vaccine is to be administered between 16–24 months. Administered via injection. This vaccine offers protection against Japanese encephalitis.

Potential, side effects: Redness, pain, tenderness, and swelling around the area of the vaccine injection.

Diphtheria, pertussis and tetanus (DPT): Booster 1. The vaccine offers protection from diphtheria, pertussis and tetanus.

The first of two doses of the DPT vaccine. The second dose is given to the child between 5 and 6 years old. Administered via injection.

Potential side effects: Fever, irritation, loss of appetite, redness, and swelling around the area of the vaccine injection.

Oral polio vaccine–booster. This vaccine protects against the poliovirus. This is a single-dose vaccine. Administered orally.

Potential side effects: There are no common side effects associated with this vaccine.

BAQ: Give an account of vaccines given to a child 10 years after birth.

Ans: Vaccination: 10 years after birth

Diphtheria, pertussis and tetanus (DPT)-Booster 2. The vaccine offers protection from diphtheria, pertussis, and tetanus.

The second of two doses, the DPT vaccine is to be given to the child when they are 5–6 years old. Administered via injection.

Potential side effects: Fever, vomiting, loss of appetite, redness and swelling around the area of the vaccine injection.

BAQ: Give an account of vaccines given to a child 6 weeks after birth.

Ans: Vaccination: 10 years after birth

Tetanus and adult diphtheria (Td). The vaccine provides protection against tetanus which can be contracted through infected cuts or wounds with the spores of the bacterium *Clostridium tetani.* It is a single-dose vaccine. Administered via injection.

Potential side effects: Mild fever, headache, nausea, diarrhea, pain, redness, and swelling around the injection area.

BAQ: Give an account of vaccines given to a child 16 years after birth.

Ans: Vaccination: 16 years after birth

Tetanus and adult diphtheria (Td). The vaccine provides protection against tetanus which can be contracted through infected cuts or wounds with the spores of the bacterium *Clostridium tetani.* It is a single-dose vaccine. Administered via injection.

Potential side effects: Mild fever, headache, nausea, diarrhea, pain, redness, and swelling around the injection area.

COVAXIN or COVISHIELD: The vaccine protects against COVID-19. Administered via injection.

Potential side effects: Mild fever, headache, nausea, diarrhea, pain, redness, and swelling around the injection area.

Booster dose: Four weeks after the first vaccination shot of COVAXIN and six weeks after the first vaccination shot of COVISHIELD.

OR

Oxford astrazeneca vaccine: The vaccine protects against COVID-19. Administered via injection.

Potential side effects: Mild fever, headache, nausea, diarrhea, pain, redness, and swelling around the injection area.

Booster dose: Four weeks after the first vaccination.

For any immediate health concerns, it is necessary to consult a local doctor or the health center in the community.

VACCINATION OF ADULTS

BAQ: Give an account of vaccines given to adults ages 19–49 years.

Ans: All adults aged 19 to 49 years should make sure that they are up-to-date on the following vaccines:

Chickenpox vaccine (varicella)

COVID-19 vaccine

Flu vaccine (influenza)

Hepatitis B vaccine

HPV vaccine (human papillomavirus)

MMR vaccine (measles, mumps, and rubella)

Tdap vaccine (tetanus, diphtheria, and whooping cough) or Td (tetanus, diphtheria).

BAQ: Give an account of vaccines given to all adults ages 50–64 years.

Ans: All adults age 50 to 64 years should make sure that they are up-to-date on the following vaccines:

- COVID-19 vaccine
- Flu vaccine (influenza)
- Shingles vaccine (zoster)
- Tdap (tetanus, diphtheria, and whooping cough) or Td (tetanus and diphtheria).

BAQ: Give an account of vaccines given to ages 65 and older.

Ans: All adults age 65 and older should make sure they are up-to-date on the following vaccines:

- COVID-19 vaccine
- Flu vaccine (influenza)
- Pneumococcal vaccine
- Shingles vaccine (zoster)
- Tdap (tetanus, diphtheria, and whooping cough) or Td (tetanus and diphtheria).

Competency achievement: The student should be able to:

PE19.4: Define cold chain and discuss the methods of safe storage and handling of vaccines

LAQ: What is a cold chain? Discuss the methods of safe storage and handling of vaccines.

Ans: A cold chain is a temperature-controlled supply chain. An unbroken cold chain is very important for an uninterrupted series of storage and distribution activities of vaccines, which maintain their good quality at a given temperature range.

The purpose of the vaccine "cold chain" is to maintain product quality from the time of manufacture until the point of administration by ensuring that vaccines are stored and transported within WHO-recommended temperature ranges (Table 18.1).

Table 18.1: Vaccine storage and handling

Vaccine	Where to store	Temp. range	Diluent storage temperature	Diluent temp
All DTaP vaccines	Refrigerator Do not freeze or expose to freezing temperatures	2°–8°C	Refrigerator	2°–8°C
Hib vaccines	Refrigerator Do not freeze or expose to freezing temperatures	2°–8°C	Refrigerator	2°–8°C
Hep A	Refrigerator Do not freeze or expose to freezing temperatures	2°–8°C	No diluent	–
Hep B	Refrigerator Do not freeze or expose to freezing temperatures	2°–8°C	No diluent	–
MMR	Refrigerator or freezer	–	–50° – + 8° C	2°–8°C
Gardasil (On HPV)	Refrigerator	2°–8°C	No diluent	–
Covaxin	Refrigerator or freezer		Refrigerator	2°–8°C
Covishield	Refrigerator or freezer		Refrigerator	2°–8°C
Pfizer-BioNTech	Ultracold freezer		Ultracold freezer	–90° and –60°C
Moderna COVID-19 vaccine	Ultracold freezer		Ultracold freezer	–90° and -60°C

BAQ: Give an account of methods of safe storage and handling of vaccines:

Ans: It is necessary to develop and maintain written standard operating procedures (SOPs) for up-to-date storage and handling of various types of vaccines. SOPs should be reviewed by all staff members and updated annually by the vaccine coordinator.

SOPs should contain plans and information for the following three main areas:

1. General information: It includes contact information for vaccine manufacturers, equipment service providers, and important facility staff, as well as job descriptions, information on regularly used forms, and staff training requirements.
2. Routine storage and handling: It includes information on all the various aspects of vaccine inventory management, from ordering vaccines to monitoring storage conditions.
3. Emergency vaccine storage, handling, and transport: Description of outline steps to be taken in the event of power failure, equipment malfunctions, natural disasters, or any other emergencies that might compromise vaccine storage conditions.

Competency achievement: The student should be able to:

PE19.5: Discuss immunization in special situations: HIV-positive children, immunodeficiency, preterm, organ transplants, those who receive blood and blood products, splenectomized children, adolescents, and travellers

IMMUNIZATION IN SPECIAL SITUATIONS

Q: Write a brief note on the general approach to immunization in special situations.

Ans: For practical considerations, persons with immunocompromising conditions may be divided into three groups:

1. Persons who are severely immunocompromised not as a result of HIV infection.
2. Persons with HIV infection
3. Persons with conditions that cause limited immune deficits (e.g. asplenia, renal failure) that may require the use of special vaccines or higher doses of vaccines but that do not contraindicate the use of any particular vaccine.

Determination of degree of immunosuppression

Individuals with severe immunosuppression: These are patients who receive cancer chemotherapy and radiotherapy, in the first two months following solid organ transplantation. Patients who have received high-dose corticosteroids for a long term. Individuals who receive biological immunomodulatory agents. Children with human immunodeficiency virus (HIV) with CD4-T lymphocyte count below 15%. Patients in whom hematopoietic stem cell transplantation has been performed.

Individuals with mild immunosuppression: These are patients who receive low-dose methotrexate (MTX: ≤0.4 mg/kg/week), azathioprine ≤3 mg/kg/day, 6-mercaptopurine ≤1.5 mg/kg/day for maintenance chemotherapy for cancer. Individuals who have received steroids for a period shorter than 14 days and at a low dose (<20 mg). Asymptomatic patients with HIV with a CD4-T lymphocyte count of 15–24%, splenectomized children and adolescents.

BAQ: Give information on immunization in the following special situations:

1. Vaccination for travellers and

2. Vaccination for diabetes mellitus patients.

Ans:

1. **Vaccination for travellers:**
   A. WHO emphasizes that all domestic and international travellers should be immunized to date with routine vaccinations.
   B. Travellers should be advised to check that they have been fully vaccinated

against the following diseases: Tetanus, pertussis (whooping cough), measles, rubella, mumps, poliomyelitis, and diphtheria, before starting their travel.

C. Non-immunized or incompletely immunized travellers should be offered the routine vaccinations recommended in their national immunization schedules, in addition to those needed for international travel (e.g. yellow fever vaccine).

D. The CDC website (www.cdc.gov/travel) has detailed information about immunizations and other precautions for travellers to other countries. Many immunizations should be received at least 1 month before travel. It is necessary to carry an immunization record when one plans to travel to other countries.

2. **Immunization in diabetes mellitus patients:** Patients with long-term diabetes mellitus may have renal, cardiovascular, and other end-organ dysfunctions. One-time pneumococcal vaccination and annual influenza and COVID-19 vaccinations are recommended for these patients.

The pneumococcal vaccine is safe and effective for these patients and does not interfere with insulin levels or blood glucose control. Patients receiving either insulin or oral antidiabetic agents respond normally to influenza vaccination without interference with diabetic control.

BAQ: Give information on immunization in the following special situations:

1. Immunization for severely immunocompromised, non-HIV-infected persons, and
2. Immunization for HIV patients.

Ans:

1. **Immunization for severely immunocompromised, non-HIV-infected persons:** Severe immunosuppression not associated with HIV can be the result of leukemia, lymphoma, congenital immunodeficiency, generalized malignancy, or therapy with alkylating agents, radiation, antimetabolites, or large amounts of corticosteroids. These patients should not be administered live vaccines.
2. **Immunization for HIV patients:** There are no vaccines to prevent or treat HIV. However, patients with HIV can benefit from vaccines against other diseases. The following inactivated vaccines are recommended for all people with HIV:

   Patients up to age 26: Human papillomavirus (HPV)

   Single inactivated vaccine on the following diseases: Diphtheria, influenza (flu); hepatitis B; meningococcal disease; pneumonia; tetanus, and pertussis

   Every 10 years, a repeat inactivated vaccine against tetanus and diphtheria is also recommended.

   Inactivated vaccines related to diphtheria pertussis and tetanus (DPT): Booster 2. The vaccine offers protection from diphtheria, pertussis, and tetanus.

Additional vaccines may be recommended for a person with HIV based on the age of the person, history of previous vaccinations, risk factors for a particular disease, or certain HIV-related factors.

Inactivated versions of vaccines are recommended and the **live (replicating) vaccines** are **contraindicated** in all HIV-positive adults and children.

BAQ: Give information on immunization in the following special situations:

1. Immunization for HIV children and
2. Immunization during pregnancy.

Ans:

1. Immunization for HIV children: Along with DPT, a single dose of PCV13 (pneumococcal conjugate vaccine) should be routinely administered to children with HIV infection aged 6 years through 18 years who did not previously receive a dose of PCV13 before age 6 years.

Inactivated versions of vaccines are recommended and the **live (replicating) vaccines** are **contraindicated** in all HIV-positive adults and children.

Vaccines are generally safe for people with HIV. However, live attenuated vaccines can potentially cause an infection in people with HIV.

2. Immunization during pregnancy: CDC recommends that pregnant women get two vaccines during every pregnancy: The inactivated flu vaccine (the injection, and not the live nasal flu vaccine) and the Tdap vaccine.

   To maximize the maternal antibody response and passive antibody transfer to the infant, the national immunization schedule in India recommends 2 doses of tetanus toxoid (TT).

   The first dose of tetanus toxoid should be administered as soon as pregnancy is detected, and the second dose of tetanus toxoid is administered after 4 weeks.

If a mother received 2 TT doses in the last pregnancy and the mother gets again pregnant within 3 years, then only one booster dose of TT is recommended.

Women in the second and third trimesters of pregnancy are at increased risk for hospitalization from influenza. Therefore, routine inactivated influenza vaccine is recommended for all women who will be pregnant during influenza season.

The hepatitis B vaccine is recommended for pregnant women at high risk.

The hepatitis A vaccine is recommended for pregnant women if she is exposed to hepatitis A.

Live vaccines are not recommended during pregnancy since the live attenuated virus in a vaccine could cross the placenta and result in viral infection of the fetus.

BAQ: Give information on immunization in the following special situations:
1. Immunization in renal failure patients and
2. Immunization in patients of alcoholic cirrhosis.

Ans:

1. *Immunization in renal failure patients:* Patients with renal failure have an increased risk of infection with a variety of pathogens, particularly pneumococcus and hepatitis B. Inactivated versions of vaccines are recommended.

   Hepatitis B vaccination is recommended for all renal failure patients.

   Pneumococcal vaccination is recommended for all renal failure patients.

   Influenza vaccine is recommended annually before the beginning of the influenza season for persons 6 months of age or older on dialysis.

2. *Immunization in patients of alcoholic cirrhosis:* Patients with alcoholism and alcoholic liver disease have an increased incidence of infections, especially pneumonia. Many of these patients have leukopenia, decreased complement activity, chemotactic defects, and impaired cell-mediated immunity. In cirrhotic patients, portosystemic shunting can diminish the clearance of bacteria and increase the severity of infection.

   Patients with alcoholism or alcoholic liver disease should receive one-time pneumococcal and yearly influenza vaccination. Inactivated versions of vaccines are recommended.

BAQ: Give information on immunization in the following special situations:
1. Persons with splenectomy and
2. Immunization in solid organ transplants (SOT).

Ans:

1. Persons with splenectomy usually have an increased risk for fulminant bacteremia, associated with a high mortality rate.

   Polyvalent pneumococcal vaccine is recommended for all asplenic persons greater than or equal to 2 years of age.

   Quadrivalent meningococcal polysaccharide vaccine also should be recommended to asplenic children greater than or equal to 2 years of age.

Immunization with the Hib vaccine should be initiated in infancy at the same dosage and schedule as recommended for healthy children.

2. Immunization in solid organ transplants (SOT): Solid organ transplant recipients need immunization that certainly decreases the risk of vaccine-preventable diseases.

   Organ transplant candidates should complete the recommended full vaccination schedule as early as possible during the courses of underlying disease because patients with end-stage liver or renal disease have reduced immune response to the vaccine. Live attenuated vaccines are generally contraindicated after transplantation.

   Recommended vaccines include pneumovax, hepatitis A and B, influenza, and tetanus-diphtheria**.**

BAQ: Give information on recommended vaccinations for health workers.

Ans: For the health workers who work directly with patients or handle material that could spread infection, it is necessary to get appropriate vaccines to reduce the chance to get or spread vaccine-preventable diseases. All healthcare workers should make sure they are up to date on the following vaccines:

- COVID-19 vaccine
- Chickenpox vaccine (varicella)
- Flu vaccine (influenza)
- Hepatitis B vaccine

Meningococcal vaccine, particularly for laboratory workers who work with *Neisseria meningitidis*

MMR vaccine (measles, mumps, and rubella)

Tdap (tetanus, diphtheria, and whooping cough) or Td (tetanus and diphtheria)

Healthcare workers should make sure that they are up-to-date on any vaccines routinely recommended for them based on age or other factors.

Other vaccines recommended are as follows: HPV vaccine (human papillomavirus) is recommended for adults ages 18 through 26 years and adults ages 27 through 45 years. The shingles vaccine (zoster) is recommended for all adults 50 years of age and older.

Chapter

19

Medical Biochemistry Laboratory Basic Requirements, Principles and Procedures

NOTE

Figures used in this chapter are only for the education of students. Students are not expected to draw or display these while answering the questions. Unless a specific question is asked by the examiner, which may require a neat figure.

SPQ: Short answer question: Marks: 2

BAQ: Brief answer question: Marks: 4 or 5

LAQ: Long answer question: Marks: 9 or 10

MCQs: Marks: 0.5 each

INTRODUCTION

Various types of instruments are used in the biochemistry laboratory, and their working is based on sophisticated techniques. Advancing technology provides ample opportunities to make laboratory testing more accurate, fast, reliable, and cost-effective. If the test results are to be useful to the physician in the diagnosis and treatment of disease, the tests must be performed as accurately as possible with a short testing time. This requires the use of good instrumentation and sound analytical methods. Following are the various instruments and techniques used in the clinical chemistry section of a biochemistry laboratory:

Competency achievement: The student should be able to:

BI11.19: Outline the basic principles involved in the functioning of instruments commonly used in a biochemistry laboratory and their applications

SAQ: What is distilled water and deionized water? Which equipment is used for the preparation of distilled water and deionized water?

Ans: Distilled water and deionized water are pure forms of water, free from ions. These types of water are used for the preparation of reagents and also for performing laboratory tests. Distilled water is obtained by using distillation apparatus (Fig. 19.1) and deionized water is obtained by using deionizer (Fig. 19.2).

SAQ: What are the principles on which the working of distillation apparatus and deionizer are based?

Ans: Distillation apparatus: Tap water is passed through tubes to the still, which is fitted with a heating coil. When heating coils are heated, water boils and pure water vapors get condensed while passing through exit tubes and distilled water is obtained.

Deionizer: Tap water is passed through specific resin columns in the deioniser. Impurities in water are adsorbed on the resins and the exit tube collects the deionized water. Distilled water is stored in a 5-liter container using a tight cap.

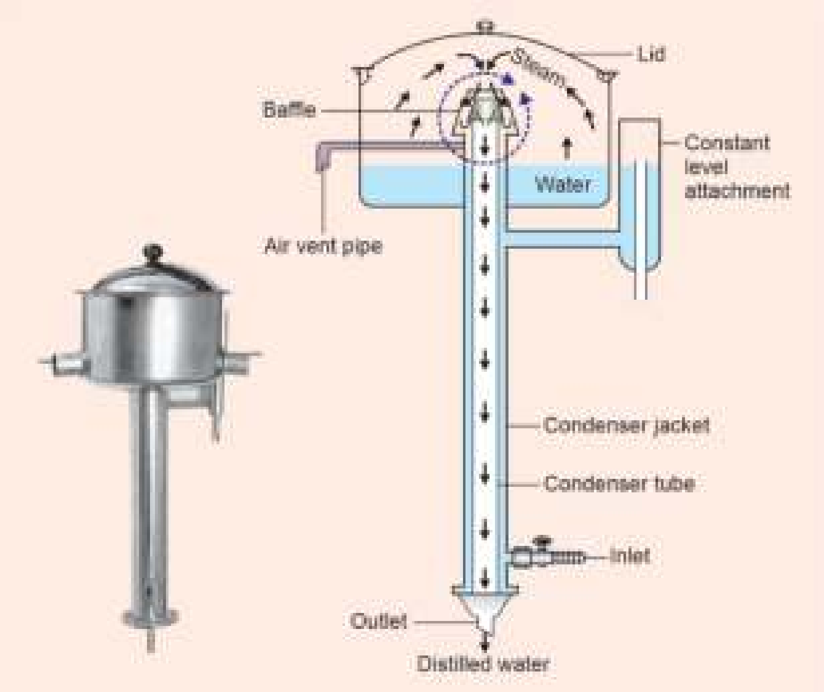
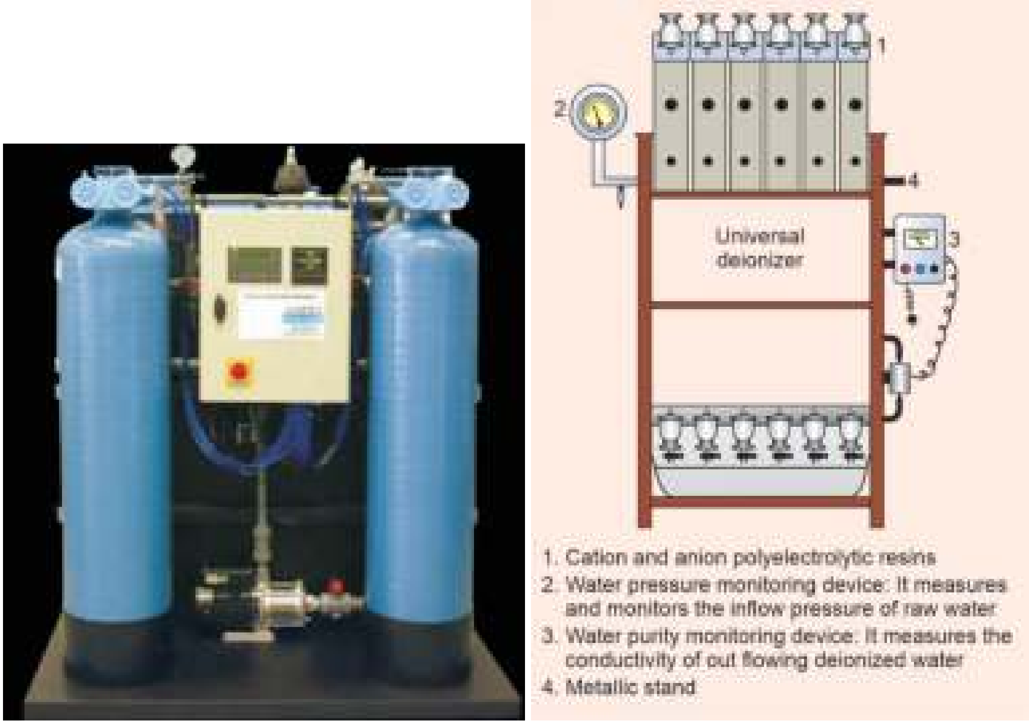

Lid
Steam
Baffle
Constant level attachment
Water
Air vent pipe
Condenser jacket
Condenser tube
Inlet
Outlet
Distilled water

Fig. 19.1: Distillation apparatus


1
2
3
4
Universal deionizer

1. Cation and anion polyelectrolytic resins
2. Water pressure monitoring device: It measures and monitors the inflow pressure of raw water
3. Water purity monitoring device: It measures the conductivity of out flowing deionized water
4. Metallic stand

Fig. 19.2: Deionizer

BAQ: Enumerate five basic types of glassware required in a biochemistry laboratory.

Ans: The following are the basic types of glassware used in a biochemistry laboratory (Figs 19.3–19.7):

1. Test tubes
2. Pipettes
3. Flasks
4. Measuring cylinder and
5. Bottles

1. **Test tubes:** 15 mm (diameter) × 125 mm (length)
   Centrifuge tubes: 10 mm (diameter) × 100 mm (length) or a conical tube
2. **Pipettes:** 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml
3. **Flasks:** Flat bottom or Erlenmeyer (conical): 100 ml, 250 ml, 500 ml.
4. **Measuring cylinders:** 100 ml, 500 ml, 1 liter
5. **Bottles:** 50 ml, 100 ml, 500 ml.

BAQ: What is a push-button pipette? How these are used and what are the advantages of the use of a push-button pipette?

Ans: For pipetting and dispensing specimens such as blood, serum, plasma, CSF, urine, etc., and also various types of reagents, it is very safe to use a push button pipette, since the user does not come in contact with infectious specimens, materials as well as corrosive reagents (Figs 19.8A, 19.9).

SAQ: What is a cyclomixer?

Ans: When a reagent is dispensed in a test tube and a specimen is added, adequate mixing of reagent and specimen is necessary. It is achieved by using a cyclomixer (Fig. 19.8B).

BAQ: Describe an electronic digital balance concerning the following points:

1. Uses
2. Components
3. The principle on which working is based
4. Care and maintenance.

Ans:

1. **Uses:** Electronic digital balance is used to accurately weigh chemical powders such as sodium chloride, glucose, etc.
2. **Components:** The electrical balances consist of three basic component systems: (a) A null detector, (b) an electromagnet, (c) A read-out device.
3. **Principle:** In an electronic balance, initially, the system is in a null position when weight (or chemical) is not placed on the single pan (Fig. 19.10). Or zero adjustment (null position) is made using a button (on RHS of Fig. 19.10). When a substance (e.g. chemical) is placed, the null

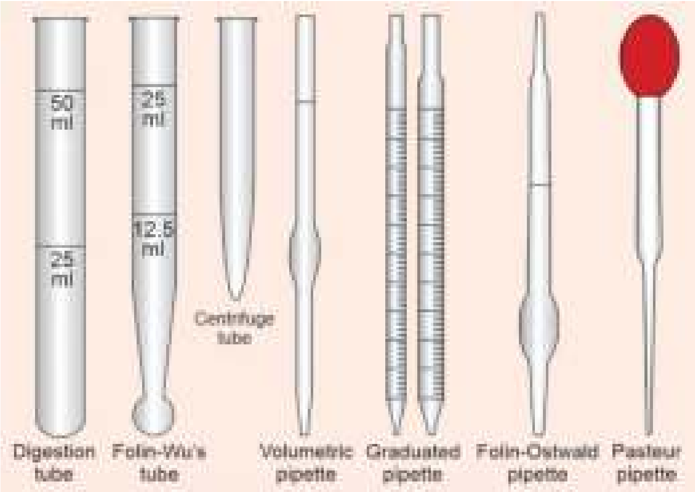


Fig. 19.3: Various types of pipettes

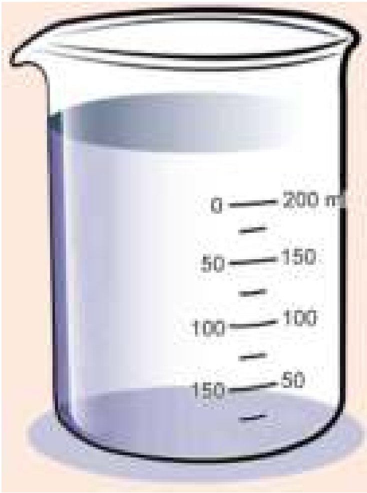


Fig. 19.4: A beaker

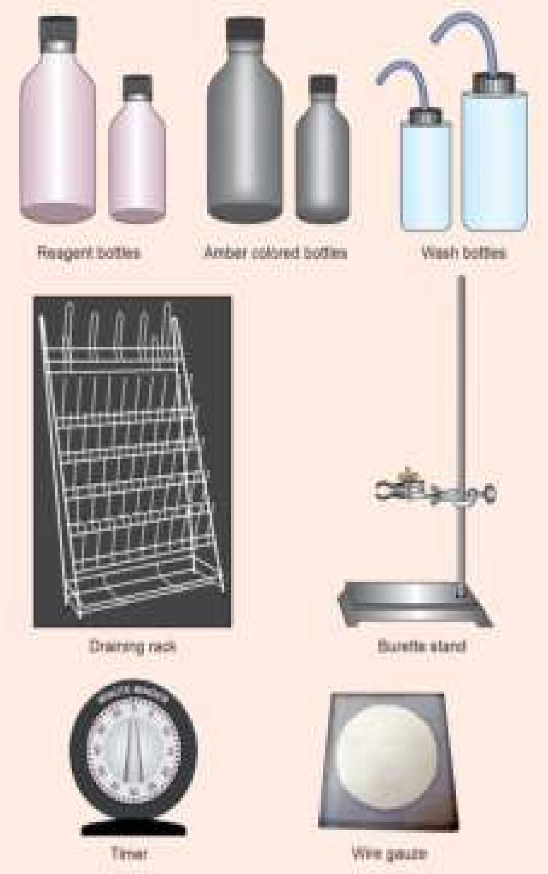


Fig. 19.5 Various requirements used in the laboratory

position is disturbed. The disturbed force is directly proportional to the electronic force, which is expressed in the digital figures on the scale indicating the weight of the substance.

4. **Care and maintenance:** The balance should be cleaned after use and a plastic cover is placed on to prevent dust accumulation.

BAQ: Describe a centrifuge for the following points (Figs 19.11A and B):

1. Uses
2. Components
3. The principle on which working is based
4. Care and maintenance.

Ans:

1. **Uses:** The centrifuge is used in a laboratory for various purposes, such as:

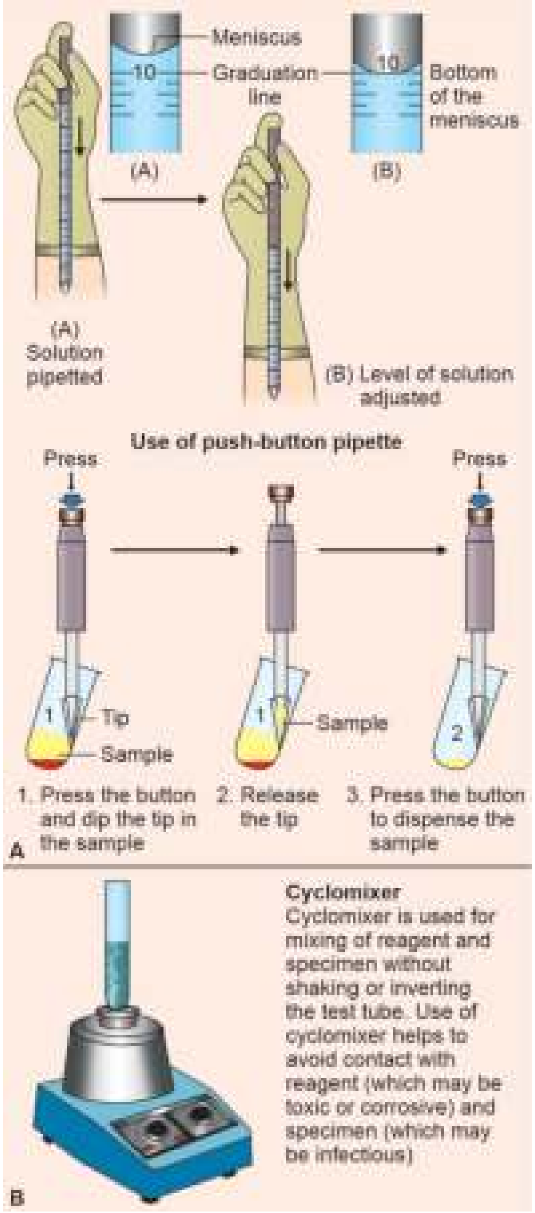
250
200
150
100
50

Fig.19.6: Measuring cylinder

Fig. 19.7: Round bottom and conical flasks

- The separation of serum or plasma from red blood cells
- Separation of sediment in urine
- Separation of protein-free filtrate
- Washing of red blood cells with normal saline
- Separation of antigen-bound fraction or antibody-bound fraction from the free fraction in immunoassays.

2. **Components:**
   A. The centrifuge head (rotor) with tubes or cups with cushions (rubber pads) at the bottom inside the cups or tubes
   B. Motor drive assembly (a commutator type driving motor)
   C. The chamber which encloses the above-mentioned internal parts
   D. A power switch


Meniscus
10
Graduation line
10
Bottom of the meniscus
(A)
(B)
(A) Solution pipetted
(B) Level of solution adjusted
Use of push-button pipette
Press
Press
1
Tip
Sample
1
Sample
2
1. Press the button and dip the tip in the sample
2. Release the tip
3. Press the button to dispense the sample
A
B

Cyclomixer

Cyclomixer is used for mixing of reagent and specimen without shaking or inverting the test tube. Use of cyclomixer helps to avoid contact with reagent (which may be toxic or corrosive) and specimen (which may be infectious)

Fig. 19.8: (A) Use of push-button pipette; (B) Use of cyclomixer

E. Speed control and the additional parts
F. A timer
G. A tachometer (to read the RPM of the machine)
H. Graphite brushes

3. **Principle:** The working of a centrifuge is based on the principle of centrifugal force, which acts on a substance in circular motion, towards the periphery.

Fig. 19.9: 8 Push button pipettes

Fig. 19.10: Digital balance

4. **Care and maintenance (good safe laboratory practice):**
   A. Place the centrifuge on a firm base.
   B. Before the centrifugation, the centrifuge tubes and the cups or tubes of the centrifuge should be balanced properly. Do not run the centrifuge with buckets or tubes (or cups) missing from the unit.
   C. The chamber should be kept clean. All the spills should be cleaned immediately since they may contain biohazardous materials (chemical, microbiological or radioactive).
   D. Always make sure that the cover is closed while the centrifuge is operating. Never open the chamber until the rotor has come to a complete stop.
   E. Place a plastic cover on the centrifuge when not in use.

BAQ: What is the difference between RPM and RCF? Explain with an example.

Ans: The usual expression of RPM (revolutions per minute) gives only the centrifugation

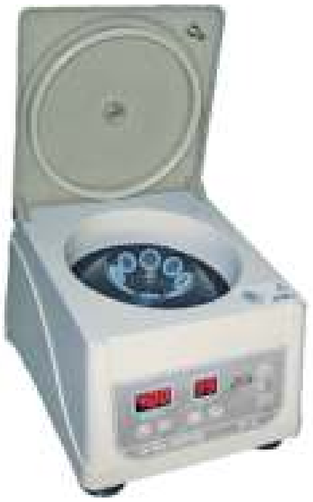


Fig.19.11A: Centrifuge

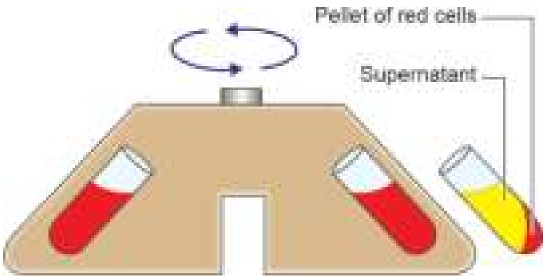


Fig.19.11B: Separation of plasma from cells from blood

speed and does not express the centrifugal force. A better expression of forces generated by centrifuge is RCF (relative centrifugal force) which is calculated by considering the speed of rotation (RPM), and the radius from the center of rotation. RCF is expressed as some number times gravity i.e. some number × g.

$$RCF = R \times (RPM)^2 \times 118 \times 10^{-7}$$

For example, 1,500 RPM and 1,500 RCF are two different values. RCF is a function of both RPM and the rotating radius of the rotor (R). Therefore, RCF is a quantitative number, while RPM is only a relative number.

BAQ: Describe a water bath concerning the following points:

1. Uses
2. Components
3. The principle on which working is based
4. Care and maintenance.

Ans:

1. **Use:** The water bath is used to carry out various chemical reactions at specific temperatures, depending upon the requirement of an experiment. The temperature of the water bath is controlled by a thermostatic arrangement. The various uses of a water bath are listed below:

Table 19.1: Uses of constant temperature water bath

Use	Temperature, °C
Determination of serum enzymes	37°
Enzymatic determinations of glucose, urea, cholesterol, triglycerides etc.	37°
Serological determinations	56°
Saponification	60–70°

2. **Components** (Fig. 19.12):
   - A. The container is generally made up of a heavily nickel-plated tank of about 20–30 liters capacity.
   - B. The heating is done by the strip heater clamped under the tank.
   - C. The temperature is controlled by a thermostat and regulated by a control knob.

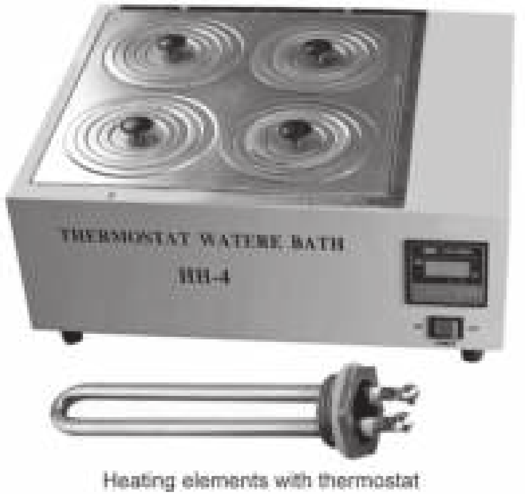


Fig. 19.12: Water bath and heating element

   D. The temperature can be recorded by a thermometer.
3. **Principle:** When electricity is passed through the heating coils, electrical energy is converted to heat energy. The temperature is controlled by a thermostat.
4. Care and maintenance of a water bath (safe laboratory practice):
   A. The water bath should be sufficiently filled with water before use.
   B. Do not forget to put off the main switch after use.
   C. Cover the water bath when not in use.

BAQ: Describe a pH meter for the following points: (1) Uses, (2) Components (3) The principle on which working is based (4) Care and maintenance.

Ans:

1. **Uses:** pH meter is used to determine the pH of a buffer solution and buffered substrate (Fig. 19.13).
2. **Components:** The following are the important components of a pH meter:
   A. *Glass electrode:* It consists of a very thin bulb about 0. 1 mm thick blown onto a hard glass tube of high resistance. The bulb contains 0. 1 mol/liter HCl connected to a platinum wire via a silver–silver chloride combination.
   B. *Calomel electrode:* It consists of a glass tube containing saturated KCl connected to platinum wires through mercury-mercurous chloride.

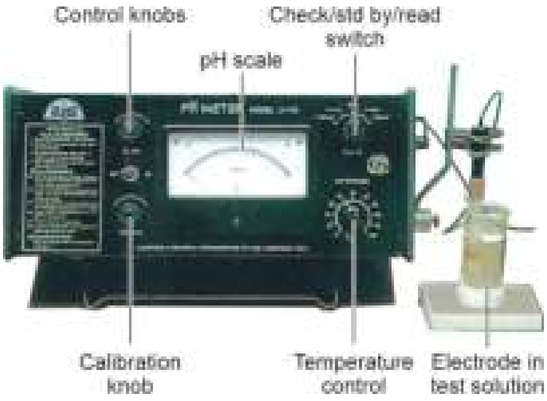


Fig. 19.13: A pH meter

   Both these electrodes could be presented in a combined form (Fig. 19.13)
   C. *Scale:* It is divided from pH 0 to 14. The reading of the pH of a solution appears on the scale.
   D. Various knobs on the pH meter for calibration and reading of pH.
3. **Principle:** When the pair of electrodes or a combined electrode (glass electrode and calomel electrode) is dipped in an aqueous solution, a potential is developed across the thin glass of the bulb (of glass electrode). The electromotive force (EMF) of the complete cell (E) formed by the linking of these two electrodes at a given solution temperature is, therefore –

$$E = E_{ref} - E_{glass}$$

   - E_{ref} is the potential of the stable calomel electrode, which at normal room temperature (25°C ± 5°C) is + 0.250 V.
   - E_{glass} is the potential of the glass electrode, which depends on the pH of the solution under test.

   The resultant small EMF can be recorded potentiometrically by using a vacuum tube amplifier. Variations of pH with EMF may be recorded directly on the potentiometer scale graduated to read pH directly.
4. **Care and maintenance:** The electrodes must be placed in water in a beaker (Fig. 19.13). It is necessary to put a cover on the pH meter using a plastic cover.

Competency achievement: The student should be able to:
BI11.6: Describe the principles of colorimetry

Competency achievement: The student should be able to:
BI11.18: Describe the principles of spectrophotometry

BAQ: Describe a photometer for the following points:

1. Uses
2. Components

3. The principle on which working is based
4. Care and maintenance.

Ans:

1. **Uses:** A photometer is used to measure optical density (OD) means, the depth of a colored solution. When a test such as serum glucose, urea, or SGPT is performed, a specific reagent or specific reagents are used, which react with the specific component to form a colored complex. The concentration of the colored complex is directly proportional to the concentration of the component in the specimen. The depth of the colored complex is measured on a photometer or spectrophotometer. The photometric readings are compared with a known primary standard or with a quality control serum sample to find out values of serum glucose, urea, SGPT, etc.
2. The following are the basic components of a photometer:
   A. A lamp: Source of light
   B. A filter (to isolate desired part of white light)
   C. A cuvette of 1-centimeter diameter (a special glass or quartz tube to hold the colored solution).
   D. Photo cells (to convert light energy into electrical energy) and
   E. A scale (to display reading in terms of OD) (Figs 19.14 to 19.17).
3. Principle: Beer's law: It states that the optical density of a colored solution is directly proportional to the concentration of the solution in the cuvette.
   Lambert's law: It states that the optical density of a colored solution is directly proportional to the path of light, i.e. the diameter of the cuvette.

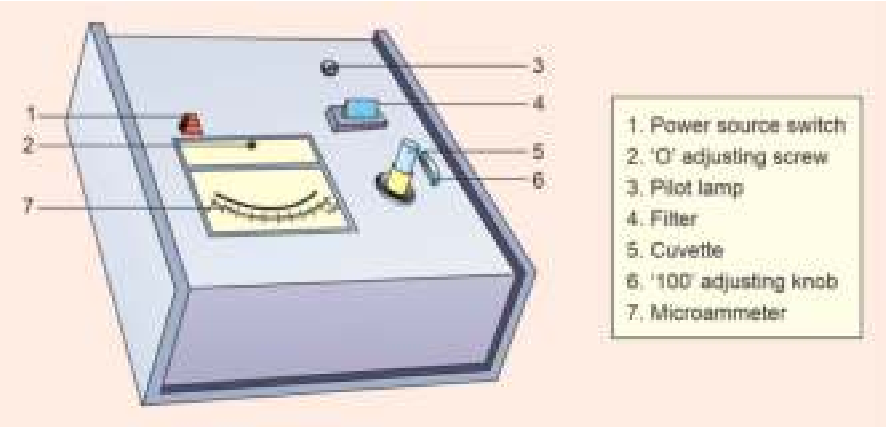


Fig. 19.14: A photometer

Fig. 19.15: A digital photometer

Fig. 19.16: A digital spectrophotometer

Fig. 19.17: Semi-autoanalyzer used for photometric readings

Since the diameter of the cuvette is the same for all the test and standard readings, only Beer's law is used.

4. Care and maintenance:
   A. Always put a plastic cover on the photometer when it is not in use.
   B. Before putting the photometer on, put a filter and cuvette filled with distilled water in their respective positions.
   C. Most of the biochemistry experiments are based on photometry; hence use the photometer very carefully.
   D. Check the sensitivity of the galvanometer occasionally by using a standard dichromate solution.

BAQ: Employing a line diagram show working of a photometer.

Ans: Following Fig.19.18, indicates the working of a photometer:

From LHS:

1. White light from the light source passes through a filter (incident light).
2. Complementary monochromatic light (band) is isolated from the filter, which passes through the colored solution.
3. Part of this light is absorbed by the colored solution (according to Beer's law).
4. The light passed through the colored solution (transmitted light) falls on the photocells. Light energy is converted to electrical energy which is directly proportional to the intensity of incident light and the scale indicates optical density reading proportional to the component under test (such as serum glucose or urea).

SAQ: What is the relation between % T (transmittance) and OD? What is the spectral range of white light? UV light and infrared light?

Ans: OD = – log T

The spectral range of white light is 380–760 nm. 1 nm (nanometer) = 10^9 meters.

UV light <340 nm and infra-red light >760 nm

SAQ: What is the importance of Beer and Lambert's law and what is the difference between a photometer and a spectrophotometer?

Ans: The working of all the photometers, spectrophotometers, and auto analyzers used in a biochemistry laboratory and also some analyzers used in microbiology, immunology, hematology, and histopathology sections are based on Beer and Lambert's law.

In the case of a photometer, a filter is used to isolate desired part (20–30 nm) of the light spectrum. In the case of a spectrophotometer, a prism and a slit are used to isolate a very narrow part of a spectrum (2–8 nm). Hence spectrophotometers are more accurate than photometers.

Competency achievement: The student should be able to:

BI11.1: Describe commonly used laboratory apparatus and equipment, good safe laboratory practice, and waste disposal

For commonly used laboratory apparatus and equipment: Refer to pages 449–454.

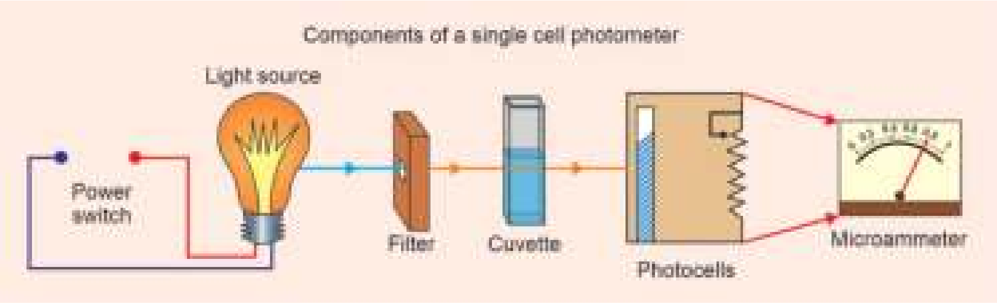


Fig. 19.18: Line diagram representing the working of a photometer

SAFE LABORATORY PRACTICE (Fig. 19.19)

BAQ: Enumerate important protocols to follow before experimenting in the biochemistry laboratory.

Ans: Before experimenting in a biochemistry laboratory, the important protocol steps are as follows:

1. Wear a laboratory coat, carry pair of gloves, a face mask, a journal, a copy of the laboratory manual (if available), a test tube marker, and a pen.
2. Get familiar with the following hazard signs and take appropriate care.
3. Get familiar with spots of fire extinguishers and fire exit routes.
4. Make sure that you have gone through all necessary immunization protocols including COVID-19 and hepatitis B.
5. Be prepared to avoid accidents and needle-stick injuries.

SAQ: What practical protocol students should follow in a laboratory?

1. Wear a laboratory coat and make appropriate use mask and hand gloves
2. Follow standard operation procedures (SOPs) for every experiment performed in a laboratory.
3. Remove the laboratory coat, wash hands and note all results of the test and prepare a report.

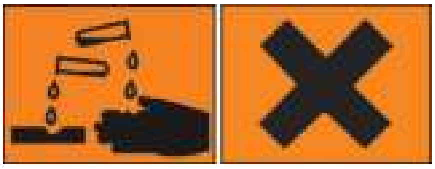
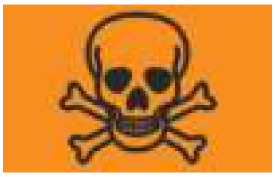
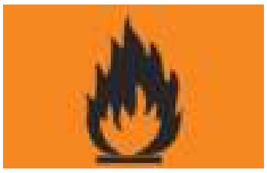
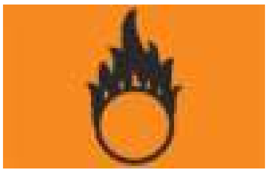
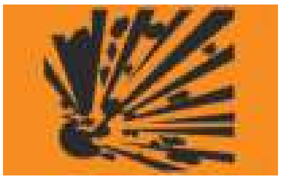
Substances	**Safety signs**
A. A corrosive substance: Destroys living tissue *Examples:* Concentrated acids, alkalies, phenol, etc.	
B. Toxic: These can be dangerous if swallowed or absorbed through skin or inhalation Examples: Potassium cyanide, ninhydrin	
C. Flammable hazards: Flammable chemicals have low flash points. These should be kept away from the area where heating procedures are carried out *Examples:* Carbon tetrachloride, ether, chloroform, alcohol, etc.	
Oxidizing substances may not be flammable themselves but may cause a fire when brought into contact with combustible material	
D. Explosives: These are not handled routinely in a pathological laboratory but some general laboratory reagent such as dry picric acid is explosive and must be handled with extreme care.	

Fig. 19.19: Safety signs

Competency achievement: The student should be able to:

BI11.2: Describe the preparation of buffers and estimation of pH

PREPARATION OF REAGENTS AND BUFFERS (BASIC REQUIREMENTS)

BAQ: What are the basic requirements to prepare a reagent?

Ans: The basic requirements for the preparation of a reagent are as follows:

Basic requirements: For the preparation of 500 ml reagent

1. Distilled or deionized water
2. Dry chemical powder
3. Butter paper
4. 500 ml beaker
5. Measuring cylinder of 1 liter
6. Glass rod
7. Reagent bottle
8. Labels
9. Digital balance

BAQ: What are the basic requirements to prepare a reagent (For example, 500 ml)?

Ans: Standard operation procedure (SOP):

1. Put on the digital balance
2. Place appropriately folded butter paper and adjust the reading to 00
3. Add the desired weight of the chemical powder
4. Transfer the chemical powder to the beaker
5. Add about 400 ml of distilled or deionized water
6. Mix using a glass rod (or magnetic plate) till the chemical powder dissolves.
7. Transfer to a measuring cylinder and make a final volume of 500 ml
8. Mix in the beaker and store in a reagent bottle using a tight cap
9. Label the bottle with the appropriate name and date of preparation.

BAQ: Write SOP for the preparation of a phosphate buffer (M/15), pH 7.45. Quantity: 200 ml.

Ans: Requirements: Same as mentioned above. The chemicals required are as follows:

Dry chemical powders: $Na_2HPO_4.2H_2O$: M/15 weight = 2.36 g

Dry chemical powders: KH_2PO_4:M/15 weight = 1.8 g

Additional requirement: pH meter

Standard buffers: pH 7.5 and pH 4.0

Standard operation procedure

1. Weigh the chemicals as mentioned above.
2. Transfer to a 500 ml beaker
3. Add 150 ml distilled or deionized water and mix well
4. Put on the pH meter and immerse the electrode in the standard solution and adjust the pH to 7.45 by turning the knob meant for reading. Also, check the pH of another standard buffer solution (pH 4.0) **Refer to Fig. 19.13.**
5. Wash electrodes with water, dry using tissue paper and immerse them in the prepared buffer solution
6. Turn the knob meant for reading the pH
7. If pH is 7.45, store this prepared buffer in a 250 ml reagent bottle and label it.
8. If pH is not accurate, adjust it using small quantities of 1N HCl or 1N NaOH.

BAQ: What are the basic requirements to perform a biochemistry test?

Ans: The following are the basic requirements for performing a biochemistry test:

1. Specimens such as blood, plasma, serum, CSF, urine, etc.
2. Reagents for the test.
   Example: Glucose reagent, urea reagent, etc.
3. Standards
   Examples: Glucose standard (100 mg/dl), and urea standard (20 mg/dl) of known concentrations, for plasma glucose and urea determinations respectively.
4. Distilled or deionized water
5. Test tubes: 15 × 125 ml

6. Test tube stand
7. Photometer or spectrophotometer or semi-autoanalyzer.

LAQ: Give brief information on the method of collection and storage of the following specimen:

1. Blood,
2. Plasma,
3. Serum,
4. Cerebrospinal fluid (CSF) and
5. Urine (for routine tests).

Ans:

1. **Blood collection method:** Venipuncture (Fig. 19.19).

   Instructions to the patient: The patient should fast for 8–12 hours according to the specific biochemistry tests.

   For whole blood and plasma, 5–7 ml of blood is collected using a sterile syringe (Fig. 19.19). In an appropriate anticoagulated tube or a bulb.

   For serum, blood is collected in a plain tube without anticoagulant.

2. **Plasma separation:** Whole anticoagulated blood is centrifuged at 3000 RPM for 10 minutes. The upper serum layer (above red cells) is collected using a Pasteur pipette.

3. **Serum separation:** Blood collected without anticoagulant is allowed to clot for 30 minutes. Fluid separated from the clot is centrifuged at 3000 RPM for 10 minutes. The upper serum layer (above red cells) is collected using a Pasteur pipette.

   Storage of blood, serum, and plasma: In the refrigerator: 2–8°C.

   Arterial puncture (Fig. 19.20)

   For the determination of blood pH, PCO_2, PO_2, and bicarbonate, arterial blood (2 ml) is used. An arterial puncture requires considerable skill and is usually performed only by physicians or by specially trained nurses or technicians. The sites preferred for arterial puncture are the radial artery at the wrist, the brachial artery in the elbow, and the femoral artery in the groin.

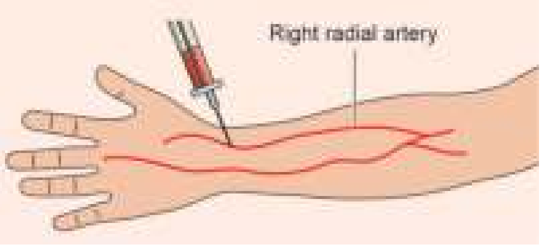


Fig. 19.20: Arterial blood collection

   Storage of arterial blood: In the refrigerator: 2–8°C.

4. **Cerebrospinal fluid (CSF)** (Fig. 19.21)

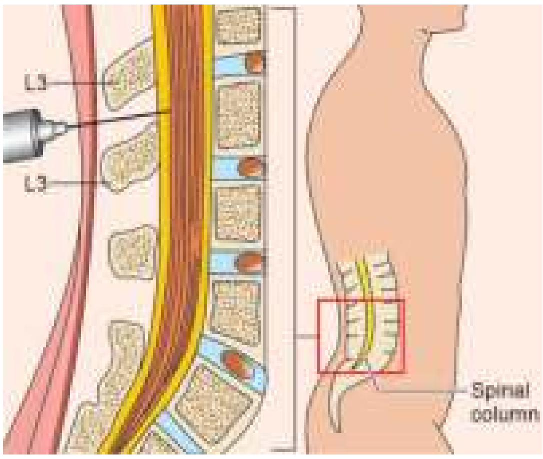


Fig.19.21: CSF collection

   CSF collection: A sterile lumbar puncture needle is inserted between the 3rd and 4th lumbar vertebra to a depth of 4–5 cm. After the withdrawal of stylet, the fluid is collected through the needle into two plain test tubes: One sterile tube for the study of microorganisms and a second plain tube for biochemistry studies.

   Storage of CSF (for routine examination): 2–8°C.

5. **Urine collection:** Type of specimen: Patient should collect first voided midstream morning urine.

   The container used for urine collection: Clean and dry wide-mouth glass or plastic bottles, with screw cap tops (capacity, about 250–300 ml)

Fig. 19.22: Urine specimen

Storage of urine: In the refrigerator: 2–8°C.

NOTE

1. For the collection of stool specimens, the patient should collect a small portion of stool in a container shown in Fig. 19.22.
2. For the collection of gastric specimen, Rehfuss or Ryles tube is introduced through mouth and specimen are collected.

SAQ: Enumerate the requirements for blood collection.

Ans: The following are the requirements for blood collection:

1. Disposable syringes and needles (of bore sizes 19, 20, and 21) or vacutainer systems.
2. Disposable lancets
3. Gauze pads or adsorbent cotton
4. Tourniquet
5. 70% (V/V) ethanol
6. Test tube (15×125 mm, medium size), without anticoagulant.
7. Needle disposal system

BASIC STEPS FOR DRAWING A BLOOD SPECIMEN

BAQ: Describe the basic steps for drawing a blood specimen.

Ans: The following are the basic steps for drawing a blood specimen:

1. Confirming, whether the patient has fasted. Some tests require the patient to fast. Such care is needed to ensure accurate results.
2. Reassuring the patient. The technician must gain the patient's confidence and assure him that, although the venipuncture will be slightly painful, it will be of short duration.
3. Positioning the patient:
    a. The patient should be made to sit comfortably in a chair and should position his arm on a slanting armrest, extending the arm straight from the shoulder, and it should not bend at the elbow.
    b. If the patient wants to lie down, let the patient lie comfortably on the back. The patient should extend the arm straight from the shoulder. For support, a pillow may be placed under the arm.
4. Checking the paperworks and tubes: The tubes and bulbs should be checked for appropriate kinds and paper labeling.
5. Selecting the vein site: For most venipuncture procedures on adults, veins located in the arm are used. The median cubital vein is the one used for the patient. If the venipuncture of this vein is unsuccessful, one of the cephalic or basilic veins may be used. The blood, however, usually flows more slowly from these veins (Fig. 19.23).

Skin Puncture Blood Collection

BAQ: Give information on blood collection by skin puncture.

Ans: In an adult or grown child, blood may be obtained by puncturing the tip of a finger or by piercing an earlobe. The skin of the palmar side of the tip of the third or fourth finger of the non-writing hand should be first cleaned by using a cotton or gauze pad saturated in 70% ethanol (or isopropanol). After evaporation of alcohol, when the skin is dry, a sharp stab is applied with a lancet. The depth of the incision should be less than 2.5 mm to avoid contact with bone. The finger should be held in such a way that gravity assists the collection of blood on the fingertip (Fig.19.24).

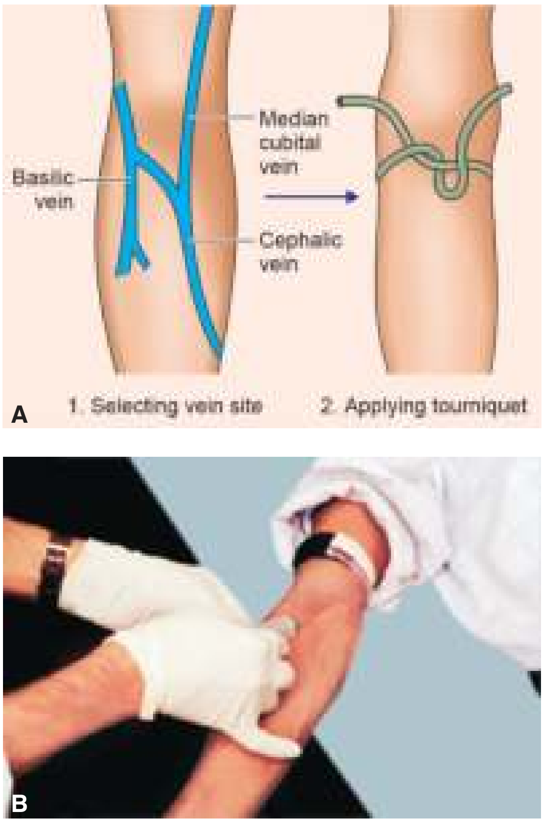


Fig. 19.23A and B: Appropriate veins for venipuncture

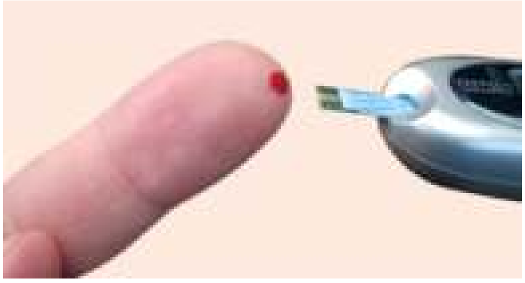


Fig.19.24: Blood collection by skin puncture

BAQ: Enumerate precautions taken while collecting blood.

Ans: The following precautions are taken while collecting blood:

The phlebotomist must be conversant with the following rules:

1. Universal precautions (UPS) means that all human blood and certain body fluids are treated as if known to be infectious for HIV, HBV, and other blood-borne pathogens.
2. Personal protective equipment (PPE) in specialized clothing or equipment is worn to protect from occupational exposure. PPE includes gloves, gowns, laboratory coats, face shields or masks, etc.
3. The laboratory request form should be dated and include a number to identify all paperwork and specimen associated with each patient.
4. The laboratory request form should provide the following information.
    - Patient's full name, age, sex, and weight (if necessary)
    - Identification number
    - List of required specific tests
    - Urgent tests: Only those tests that are required for the immediate care
    - Name of the physician ordering the test.

SAQ: What are anticoagulants? Write information on the following commonly used anticoagulants in the biochemistry laboratory concerning action and quantity: Sodium fluoride, heparin, and EDTA.

Ans: Anticoagulants are the chemicals that prevent the coagulation of blood after the collection in a tube.

Sodium fluoride

Sodium fluoride is a weak anticoagulant and prevents glycolysis by inhibiting the enzyme systems involved in glycolysis. It is used in combination with potassium oxalate. Usually, one part of sodium fluoride and three parts of potassium oxalate are mixed to prepare anticoagulated powder, and 8 mg of this powder is used to collect 2–3 ml of blood.

Heparin

It is available as sodium, potassium, lithium, and ammonium salts. It causes the least interference with tests. It prevents coagulation of blood by acting as antithrombin to prevent the transformation of prothrombin into thrombin and, thus, the formation of fibrin from fibrinogen. Most blood tubes are prepared with powdered 0.2 mg heparin for each ml of blood to be collected.

Ethylenediaminetetraacetic acid (EDTA)

Since this anticoagulant preserves the cellular components well, it is used for hematological examinations and also for glycosylated hemoglobin tests. It is used as a disodium or dipotassium salt. Dipotassium salt is preferred because it is more soluble compared to disodium salt.

EDTA prevents coagulation by binding calcium, which is essential for the clotting mechanism. It is effective at a final concentration of 1 to 2 mg/ml of blood.

BAQ: What are evacuated tubes (vacutainers)?

Vacutainers are used to collect blood by venipuncture or by finger prick method instead of conventional syringes and needles (Fig.19.25).

The evacuated tube system consists of the following components (Fig. 19.25).

1. Sterile single-use blood collection needle.
2. Holder: It is used to secure the needle during insertion into the tube stopper and subsequent venipuncture.
3. Sterile vacutainer primary tube: It is an evacuated glass tube with a rubber stopper containing a pre-measured vacuum (and anticoagulant for whole blood).

Vacutainer brand needles are specifically designed. The needle has two ends. The cannula-tubing from which the needle is made is sharpened at one end for puncture of the skin and vein. A plastic hub is at the center, which screws into the holder. At the back end is a much shorter cannula. The rear cannula has a rubber sleeve.

BAQ: Enumerate the various advantages of evacuated tube systems.

1. Evacuated system eliminates the preparation of anticoagulant-containing bulbs and tubes.
2. There is no processing of containers.
3. It minimizes hemolysis in the specimens.
4. The system is closed, and there is no possibility of blood spillage. The tubes are completely leakproof during transport.
5. Since a syringe is not required, there are fewer disposals. Only the needle is disposed of after blood collection.
6. Each vacutainer tube is designed to draw exactly the right amount of blood. Correct blood-to-additive ratio is maintained. Hence the chance of micro-clot formation and hemolysis of blood are minimum.
7. The interior of vacutainers is sterile, which prevents microbial growth.
8. An inert polyester gel barrier facilitates rapid clotting and separates serum and cells during centrifugation. Many auto-analyzers are designed to sample directly from vacutainer tubes.
9. The plasma separator tube is heparinized and contains an inert gel barrier. These tubes are mainly useful for the quick separation of plasma and the determination of electrolytes.
10. Capillary blood collection tubes are designed for the collection, transport, and processing of skin puncture blood from infants, children, geriatric, and critical care patients. Microgram tube closures reduce blood spatter upon removal.

Q: By means of a table show color, related anticoagulant, and use of evacuated tubes.

Ans:

Table 19.2: Anticoagulated bulbs or tubes for blood collection

Color	Anticoagulant	Use
Red	—	For serum
Lavender	EDTA (Na_2 or K_2)	Whole blood for CBC
Blue	Sodium citrate (liquid)	Whole blood for ESR and coagulation test
Green	Heparin	Plasma or coagulation test whole blood
Gray	Sodium fluoride	Plasma for blood glucose

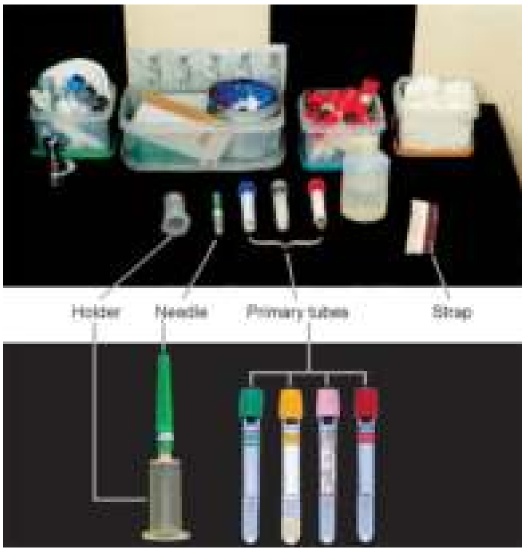


Fig. 19.25: Evacuated blood collection materials

Patient Aftercare

BAQ: Write on patient aftercare measures following venipuncture.

1. If bleeding from the puncture site continues for an unusually long time, elevate the area and apply a pressure dressing. Observe the patient closely. Check for anticoagulant and ASA (acetylsalicylic acid) type injection.
2. If the patient feels dizzy or faints, put the head down between the knees or have the patient lie flat and breathe deeply. A cool towel may be applied to the head or back of the neck. If the patient remains unconscious, notify the physician immediately.
3. Hematomas can be prevented by the following steps: Use of the proper technique, release of tourniquet before the needle is withdrawn, application of sufficient pressure over the puncture sites, and maintenance of extended extremity until the bleeding stops.

BAQ: What is the difference between serum and plasma? Write the procedure for the separation of serum and plasma.

Ans: Serum is the fluid obtained after blood clotting, by removing the clot by centrifugation and plasma is the fluid obtained by centrifuging anticoagulated blood (mainly in heparin). The serum does not contain fibrinogen, while plasma contains fibrinogen.

Separation of serum

1. Collect 5 to 7 ml of blood in a tube (which does not contain any anticoagulant).
2. Keep the tube in a slanting position and allow the blood to clot at room temperature (25°C ± 5°C). However, if blood is collected in a vacutainer tube (which contains clot-activating material), it should be kept in a vertical position at room temperature (25°C ± 5°C) for 15–30 minutes.
3. After 15 to 30 minutes, loosen the clot slowly, and by using a Pasteur pipette, transfer the separated serum into a centrifuge tube and centrifuge it at 1,500 RPM for 10 minutes.
4. Pale yellow colored serum is obtained above the packed red blood cells in the centrifuge tube.
5. Transfer it to a clean and dry test tube by using a Pasteur pipette, label it and stopper appropriately and immediately store at 2–8°C, till it is used to perform a test.

It is necessary to note the following points:

1. Never keep blood samples in the refrigerator or the incubator for clotting
2. In the case of a vacutainer tube, inadequate clotting time, improper mixing, and failure to place the tube in an upright position can lead to incomplete clot formation.

Separation of plasma

1. Collect about 5 ml of blood in a specific anticoagulant-containing tube or bulb.
2. Shake the tube (or bulb) gently to mix the anticoagulant with the blood.

   A delay in shaking the tube gently may cause partial clotting of the specimen.
3. Centrifuge at 1,500 RPM for about 10 minutes. Pale yellow-colored plasma will separate above the sedimented red blood cell pack.

4. Transfer the plasma to a clean and dry test tube, label it appropriately, and store it at 2–8° C till a test is performed on this specimen.

Specimen Rejection Criteria

BAQ: Enumerate specimen rejection criteria.

Ans: The following are the specimen rejection criteria:

1. Specimen improperly labeled or unlabeled.
2. Specimen improperly collected or preserved.
3. Specimens were submitted without a properly completed request form.
4. Specimen sample volume not sufficient for the requirement of the test protocol.
5. Patients were not prepared properly for test requirements
6. If separated, serum or plasma is grossly hemolyzed.

Hemolysis of Blood

Q: What is the hemolysis of blood? Enumerate various reasons that lead to hemolysis of blood.

Ans: Hemolysis means the liberation of hemoglobin after red blood cells have ruptured. Due to hemolysis, the serum or plasma assumes pink to red color. It is important to avoid hemolysis at every step during blood sampling, transportation, and storage because hemolysis causes a specific or nonspecific change in the measurements of many analyses.

In venipuncture, hemolysis may occur by:

1. Using a very small needle
2. Forcing the blood through the needle
3. Shaking the tube or bulb vigorously after blood collection
4. Presence of excess anticoagulant in the container (tube or bulb)
5. Centrifuging blood samples at high speed before completion of clotting
6. Freezing or thawing of blood
7. Using unclean tubes with residual detergent
8. Presence of water in the container (tube or bulb).

HOW TO PERFORM A BIOCHEMISTRY LABORATORY TEST?

BAQ: What are the basic requirements to perform a test in a biochemistry laboratory?

Ans: The following are the basic requirements to perform a test in a biochemistry laboratory:

1. Specimen (blood, plasma, serum, CSF, urine, etc.)
2. Reagents or a reagent kit (of a company that contains required reagents) (Fig. 19.27)
3. Test tubes (15 × 125 mm) with a test tube stand (Fig. 19.26)
4. Photometer or spectrophotometer or semi-autoanalyzer (Figs 19.14–19.17)

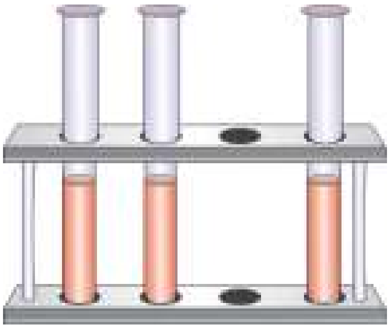


Fig. 19.26: Test tubes and test tube stand

SGPT (ALAT) KIT
QA APPROVED
CE
SGPT (ALAT) KIT

Fig. 19.27: Reagent kit

5. Water bath set at the desired temperature (Fig. 19.12)
6. Centrifuge (Fig. 19.11)
7. Cyclomixer (Fig. 19.8B)
8. Test tube markers or labels
9. Journal, notebook and pen

BAQ: What are the basic steps (SOP) of a biochemistry test?

Ans:

1. Take three test tubes and label them as T (Test), S (Standard), and B (Blank)
2. Add serum or plasma (as per SOP) in the tube labeled as "T".
3. Add standard (as per SOP) in the tube labeled as "S ".
4. Add deionized water (as per SOP) in the tube labeled "B"
5. Mix well on a vortex mixture
6. Incubate the tubes (as per SOP)
7. Take OD readings using a photometer or spectrophotometer or semi-autoanalyzer
8. Calculate the test values (as per SOP)
9. Prepare a report

BAQ: What are the units used to express values of serum, plasma, urine, and CSF components such as glucose, uric acid, enzymes, hormones, etc.?

Ans: Refer to Table 19.3:

SI Units

The units used in this textbook are SI units (The System International d' Unite's, SI). These units are based on the metric system. The SI units are being adopted by scientific laboratories throughout the world. The basic SI units used in this book are as follows:

Units used in conjunction with SI

These units are convenient to use in biochemical work and hence are used in conjunction with SI units.

1. Gram (g): The gram (g) is used as an elementary unit in association with mg and µg
   1 kg = 1000 g
   1 g = 1000 mg
   1 mg = 1000 µg
2. Liter (l or L): Although the SI unit for volume is the cubic meter (m^3), the Liter (l or L) is accepted as the convenient unit of volume.
   1 liter = 1000 ml
   1 ml = 1000 µl

Competency achievement: The student should be able to:

BI11.1: Describe commonly used laboratory apparatus and equipment, good safe laboratory practice, and waste disposal

For commonly used laboratory apparatus and equipment: Refer to pages 453–460.

WASTE MANAGEMENT

BAQ: Write a note on waste management.

Ans: Waste management is necessary for clinical laboratories because of federal and state regulations that control and restrict the

Table. 19.3: Serum, plasma components and related units

Components	Units
Glucose, urea, uric acid, creatinine, calcium, inorganic phosphorus, cholesterol, triglycerides LDL- cholesterol, HDL-cholesterol	mg/dl (100 ml)
Proteins, albumin, globulin	g/dl (100 ml)
Sodium, potassium, chlorides, bicarbonate	Milliequivalents/L (mEq/1000 ml)
T3	Nanograms/L (ng/100 ml)
T4	Micrograms/dl (100 ml)
TSH	Microinternational units/dl (mIU/ 100 ml)
SGPT, SGOT, alkaline phosphatase, LDH	International units/L (IU/100 ml)

disposal of waste generated by all medical faculties. There are three distinct kinds of waste generated in the laboratories:

1. Medical wastes: These include infectious wastes (such as human blood, body fluids, and discarded microbiological cultures) and discarded sharps (which include needles, lancets, scalpels, blades, and broken glass, capillaries), blood collection materials (syringes, vacutainers, giving and taking sets), and disposable ESR tubes.
2. Hazardous chemical waste includes corrosive, toxic, carcinogenic, ignitable, and explosive chemicals.
3. Garbage and trash include everything else except medical and hazardous wastes.

Standard waste disposal procedure

The following are the steps followed for standard waste disposal:

1. Separate medical and hazardous waste from trash and garbage.
2. Discard liquid wastes into the sewage system (except those containing heavy metals).
3. Discard trash and garbage (waste papers, cartons, thermocol, plastic, etc.) into *black* colored plastic bags.
4. Place reusable micro-tips, micro-cups, specimen containers, glassware, and plasticware in 5% hypochlorite solution, autoclave, wash, and reuse.
5. Autoclave all microbiological waste along with containers, plates, and tubes before discarding.
6. Decant blood, plasma, serum, cerebrospinal fluid, etc. in stainless steel kettles. Autoclave containers and dispose of specimens in the manure pit.
7. Flush urine and stool in the toilet, place the reusable containers in 5% hypochlorite solution, and autoclave containers, and dispose of them in the manure pit.
8. Wash the various tissues collected (in formalin) in water and send in yellow bags for incineration.
9. Store liquid radioactive waste for 3 months before decontamination and disposal.
10. Autoclave the discarded blood units from blood bank before disposal as medical waste.

It is important to note the following points:

A. Needles are destroyed by using a needle destroyer before disposal
B. The autoclave holding time is 1.5 hours at 121°C (15 lbs pressure)
C. Incineration of contaminated waste must meet with the approval of the public health and air pollution authorities as well as that of the safety officer
D. Radioimmunoassay (RIA) laboratory should follow the guidelines of the radiation protection committee.

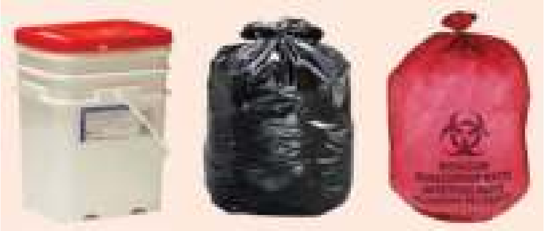


Fig. 19.28: Waste disposal containers

Competency achievement: The student should be able to:

BI11.16: Observe the use of quality control at the end of each experiment

QUALITY CONTROL

BAQ: What is quality control? Write various methods to ensure good quality control in laboratory work.

Ans: Quality control (QC) means various methods adopted by a laboratory to ensure that reliable report values are obtained so that a patient could be treated well.

QC in a laboratory ensures the physicians, quality results, and the patients' reliable reports. QC program is used to detect and correct errors before they result in a defective report, product, or service. Quality control in a clinical laboratory can be conducted by two methods: (1) Internal quality control; and (2) External quality control.

Internal quality control

Internal quality control is managed by controlling the following errors: Pre-analytical, analytical, and post-analytical.

Pre-analytical errors take place before the analysis of the specimen. Nearly 85% of all errors are attributed to pre-analytical errors. It is necessary to control pre-analytical errors.

The pre-analytical phase is comprised of specimen collection, processing, transportation, storage, and placement on an analyzer. Despite having the best quality systems for specimen testing, any error in any one of these steps in the pre-analytical phase may lead to wrong test reports. Common factors contributing to pre-analytical errors include:

1. Incorrect patient preparation
2. Selection of improper specimen collection tube (bulb or container)
3. Selection of improper anticoagulant
4. Excess of anticoagulant
5. Insufficient mixing of the specimen with anticoagulant (or specimen collection tube contents)
6. Inadequate clotting
7. Inadequate centrifugation time while separating plasma and serum
8. Hemolysis of plasma or serum
9. Improper transportation conditions and storage of specimen
10. Incorrect labeling of the collected specimen

By using appropriate measures pre-analytical errors could be minimized.

Analytical errors are attributed to the techniques such as pipetting, dispensing, and mixing and also errors committed by the equipment. By giving good training to the staff members, analytical errors could be minimized.

Post-analytical errors are attributed to miscalculations and mismanagements in the dispatch of the reports. With good training for the staff members, post-analytical errors could be minimized.

External quality control

External QC program is conducted by using quality control serum, values of components of this serum such as glucose, creatinine, etc are known. Semi- and fully-automated machines generate a QC chart known as **Levey-Jennings (L-J) chart**. At the end of every test, the generated L-J chart indicates the reliability of patient test values. QC serum values should fall within the first SD limits (Fig. 19.29).

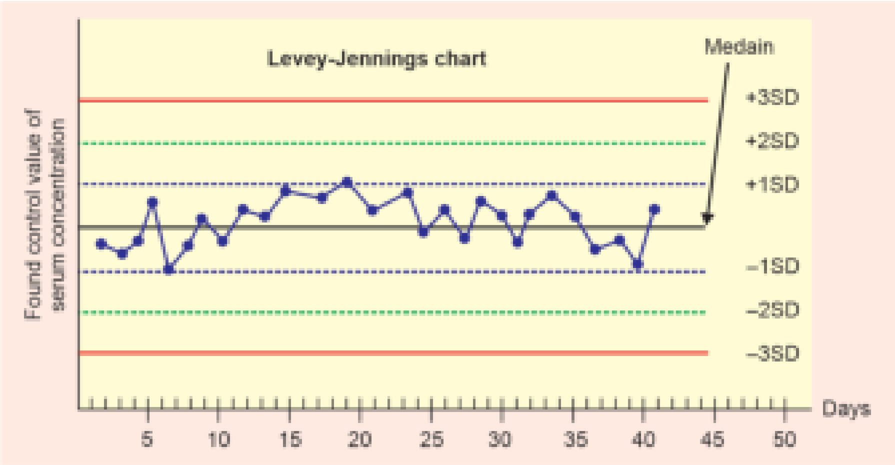


Fig. 19.29: L-J chart

Competency achievement: The student should be able to:

BI11.16: Observe the commonly used equipment and techniques used in a biochemistry laboratory

Observe the use of chromatography, electrophoresis, ELISA, paper chromatography, thin-layer chromatography, protein electrophoresis, polyacrylamide gel electrophoresis (PAGE), Electrolyte analyzer by ISE, ABL gas analyzer, radioimmunoassays, immunodiffusion, autoanalyzer, quality control in a laboratory and DNA isolation from blood and tissue (PCR technology).

REFLECTANCE PHOTOMETRY

BAQ: What is reflectance photometry and its application?

Ans: When a surface reflects light, the angle of incidence of light striking the surface is equal to the angle of reflection. A colored surface absorbs a relatively more proportion of incident light, and part of the incident light also reflects. If reflected light is measured by using a suitable monochromator and photocell-galvanometric unit, then it is possible to determine the optical density of the colored surface, and since a substance (such as glucose) is responsible for the formation of a colored surface, its concentration can also be determined (Fig. 19.30).

Use: When 5 to 10 μl of the specimen is placed on the reaction area, of a strip, the dry chemical components dissolve in the aqueous part of the sample, and the component in the sample under test (e.g. glucose) reacts with them, and the reaction area becomes colored. The optical density of the color formed can be measured by using the principle of reflectometry (Fig. 19.30).

In general, light from a light source such as tungsten, quartz–halide, tungsten–halide, or xenon lamp is collimated and strikes the colored reaction area. Diffuse reflectance is isolated by a suitable filter and detected by photomultiplier tubes. The recorded reading can be displayed in digital form.

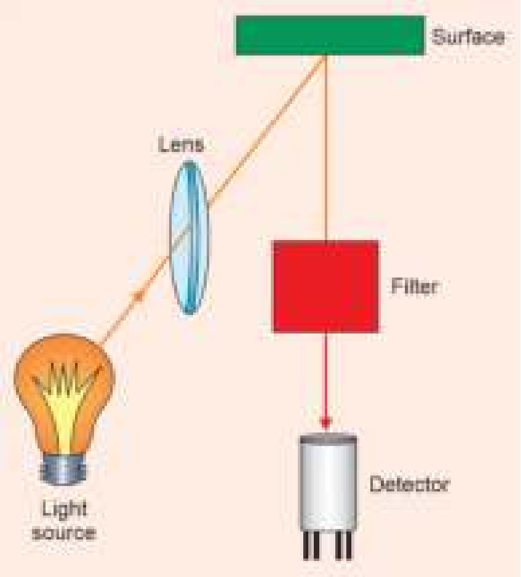


Fig. 19.30: Components of a reflectance photometer

Application of reflectance photometry: The reflectance-photometry principle is used by various glucometers (Fig. 19.31) and dry chemistry analyzers.

SELF-MONITORING OF BLOOD GLUCOSE

BAQ: Give information on self-monitoring of blood glucose by using a glucometer.

Ans: Diabetic patients require careful monitoring of blood glucose concentrations to avoid long-term hyperglycemic complications (particularly patients on insulin therapy). Point of care testing (POCT) device such as a glucometer is used to maintain glycemic control, i.e. blood glucose steady level: 70–140 mg/dl throughout the day).

Glucometers are used to determine capillary blood glucose by finger prick. Most of the glucometers are based on the reflectometry-technology. Glucometer is used as follows (Fig. 19.31):

The procedure in brief:

1. Place the glucose strip inside the glucometer (glucometer turns on).

Fig. 19.31: Glucometer

2. Prick your finger using a sterile needle.
3. Apply blood drops to a specified area on the glucose strip.
4. Blood glucose value appears on the screen.

NOTE

Capillary blood glucose is 25–35% higher than venous blood glucose.

CHROMATOGRAPHY

LAQ: What is chromatography? State the principle of chromatography, and mention the types and applications.

Ans: Chromatography is a technique through which the components of a group of similar substances present in a specimen like the serum, plasma, urine, CSF, etc. are separated on a medium such as paper, agarose gel, starch, or cellulose acetate paper.

Principle: The chromatography technique is based on a continuous distribution and redistribution of specimen components between two phases, one stationary, and the other mobile. The variable affinity of the various components towards the stationary phase and mobile phase ultimately leads to their separation. Chromatography techniques are used to separate molecules in the specimen based on differences in size, mass, shape, solubility, charge, and adsorption properties.

The following are the main types of chromatography techniques and related uses:

1. **Paper chromatography (Fig. 19.32):** In this type of chromatography, a liquid–liquid system is used in which one liquid acts as a stationary liquid phase which is supported by the cellulose fibers of a paper sheet (Whatman filter paper). The mobile phase passes along the paper sheet either by gravity feed or by capillary action. Various components in a sample get separated by their variable affinity towards the stationary and mobile phases. In most liquid–liquid systems the stationary phase is aqueous, and the mobile phase is a less polar fluid (Fig. 19.32).

   Uses (applications):

   A. Identification of characteristic amino acids excreted in aminoaciduria.

   B. Identification of sugars excreted in the urine.

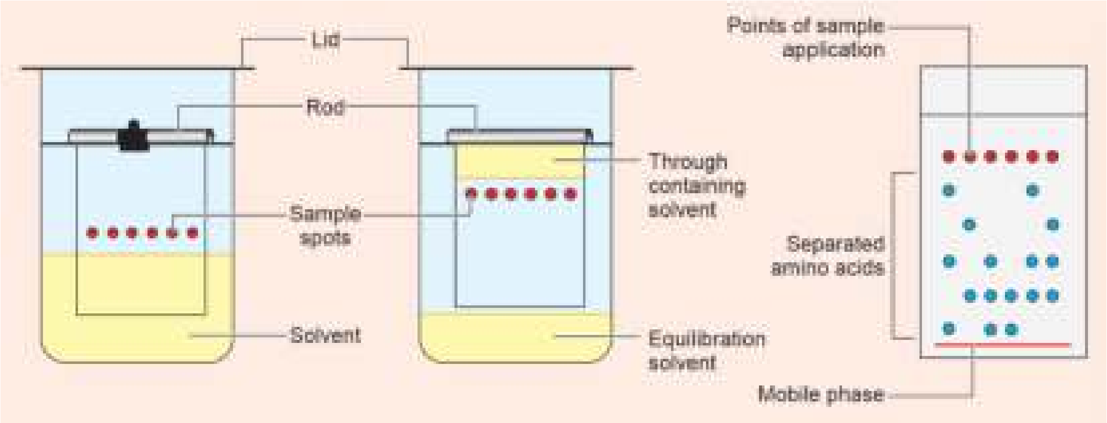


Fig. 19.32: Paper chromatography

2. **Thin-layer chromatography (TLC)**: In this type of chromatography, the stationary phase is attached to a suitable matrix. A thin layer of the matrix such as starch is coated with a plated plastic, metal, or glass. The mobile liquid phase passes across the thin-layer plate, held either vertically or horizontally by capillary action.

   Use (Application): TLC is used in the detection and quantitation of drugs, lipids, steroids, carbohydrates, nucleotides, and amino acids.
3. **Ion-exchange (column) chromatography (Fig. 19.33)**: In this type of chromatography the stationary phase is attached to a suitable matrix (inert, insoluble support). It is packed into a glass or metal column. The mobile phase in the form of an organic solvent-containing specimen is passed through the column by gravity. As the mobile phase passed down the column, the component of interest is isolated in the mobile phase and it passes through the column and other components (which are not required) are held back on the matrix column.

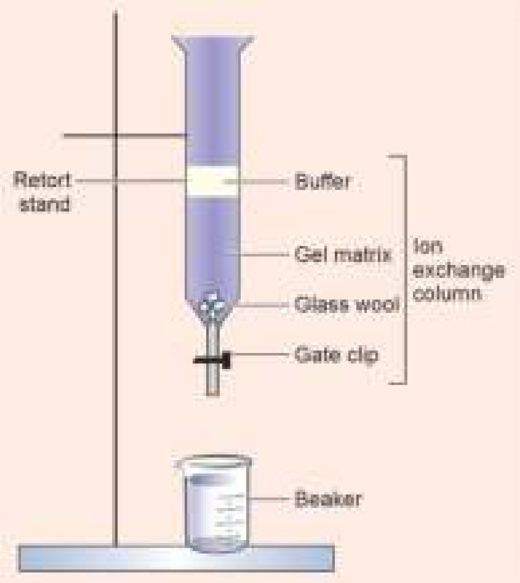


Fig. 19.33: Ion-exchange (column) chromatography

Use (application):
1. Preparation of deionized water.
2. Separation and quantitation of glycosylated hemoglobin in EDTA-blood.

ELECTROPHORESIS

LAQ: What is electrophoresis? State the principle of electrophoresis, and mention the types and applications.

Ans: Electrophoresis means the migration and separation of charged particles such as proteins in a specimen under the influence of an electric field (Figs 19.34, 19.35).

Principle: Many important biological molecules in a specimen such as proteins, peptides, proteins, nucleotides, and nucleic acid, possess ionizable groups and, therefore, at any given pH exist in solution as electrically charged species, either as anions (–) or cations (+). Under the influence of an electrical field, these charged particles (cations) migrate to the cathode (negative electrode) or (anions) move to the anode (positive electrode), depending on the nature of their net charge. The rate of movement depends on molecular weight and net charge possessed by various components in the specimen. Thus from a mixture of proteins (e.g. serum proteins), various proteins such as albumin and various globulins get separated on a specific medium.

Various types of electrophoresis: It depends on the supporting medium used to separate a mixture of components such as proteins. The following are various types of electrophoresis:

1. **Paper electrophoresis**: In this type of electrophoresis, Whatman paper (type 1 or type 3) is used as a medium.
2. **Agarose gel electrophoresis**: In this type of electrophoresis, agarose gel is used as a medium.
3. **Cellulose-acetate electrophoresis**: In this type of electrophoresis, cellulose acetate paper is used as a medium.
4. **Acryl amide gel electrophoresis**: In this type of electrophoresis, acryl amide gel is used as a medium.

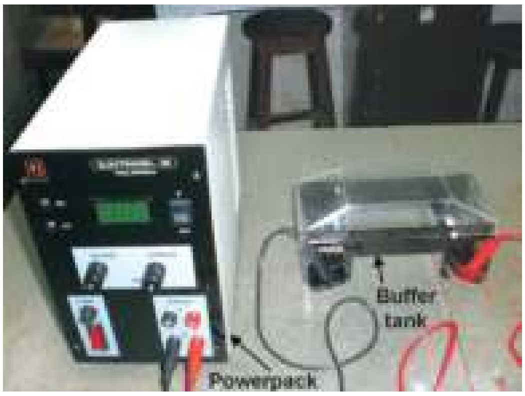

Buffer tank
Powerpack

Fig.19.34: Apparatus for agarose gel electrophoresis

Example of Electrophoresis Basic Procedure using Agarose-Gel

Procedure

1. Agarose gels are laid on glass slides
2. The glass slides are placed in the buffer tank and connected to buffer in the buffer tanks and buffer tank connected to the power pack (Fig. 19.34).
3. Serum specimens (5 µl) are applied to the agarose gel
4. Electrical current is passed for about 90 minutes
5. Gels are removed and stained using a staining solution and then destained using a destaining solution.

Results: Serum proteins get separated as follows in electrophoresis:

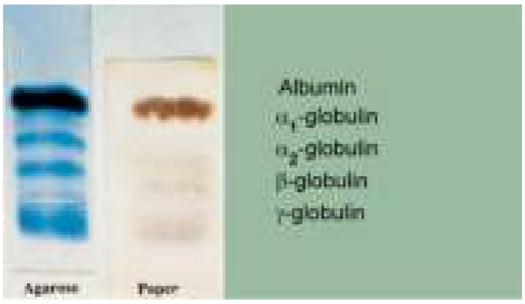

Albumin
α_1-globulin
α_2-globulin
β-globulin
γ-globulin
Agarose
Paper

Fig. 19.35: Fractionated serum proteins by electrophoresis

Uses (applications):

1. Separation of serum proteins (to examine changes in serum protein pattern in diseases). Serum proteins are separated as albumin, alpha-1 globulin, alpha-2 globulin, beta globulin, and gamma globulin.
2. Separation of serum lipoproteins (to examine changes in serum lipoprotein pattern in diseases). Serum lipoproteins are separated as chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

BAQ: Explain the basic polyacrylamide gel electrophoresis (PAGE) procedure.

Ans: Procedure:

1. Polyacrylamide gels are prepared in a glass tubes as shown in Fig. 19.36.
2. Serum samples (5 µl) are added on top of the gels.
3. These gels are connected to buffer tanks.
4. Electric current is passed for about 45 minutes.
5. Gels removed, stained, and then destained.
6. Proteins are separated on the gel (Fig. 19.36)

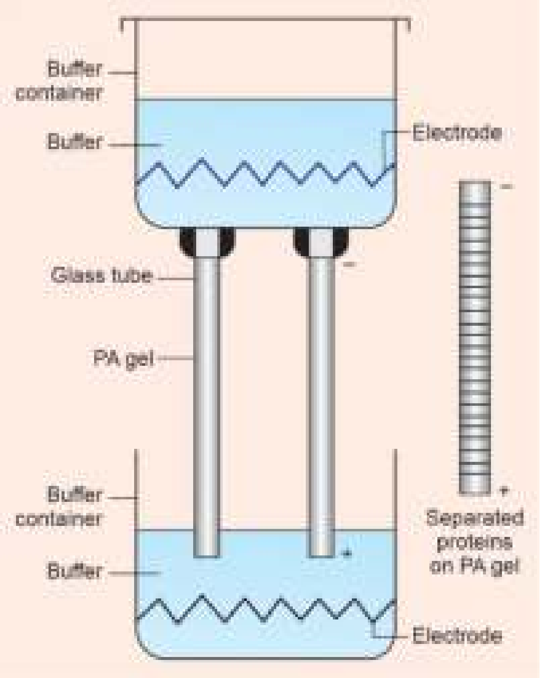

Buffer container
Buffer
Electrode
-
Glass tube
-
PA gel
+
Buffer container
Buffer
+
Separated proteins on PA gel
Electrode

Fig. 19.36: Polyacrylamide gel electrophoresis (PAGE)

ION SELECTIVE ELECTRODE (ISE) ANALYZER USE

BAQ: Explain the working of an ion-selective electrode (ISE) analyzer for the determination of serum electrolytes: Sodium and potassium.

Ans: Working of ion selective electrode (ISE) analyzer: Iron-selective electrodes specifically determine the concentration of positive ions in serum such as sodium and potassium. Sodium electrode determines serum sodium and potassium ions determine potassium ions.

Working procedure: Introduce serum to the electrodes of the analyzer (**Fig. 19.37**). Serum sample passes through two channels to sodium and potassium electrodes, respectively and readings of sodium and potassium ions are displayed on the screen of the electrolyte analyzer.

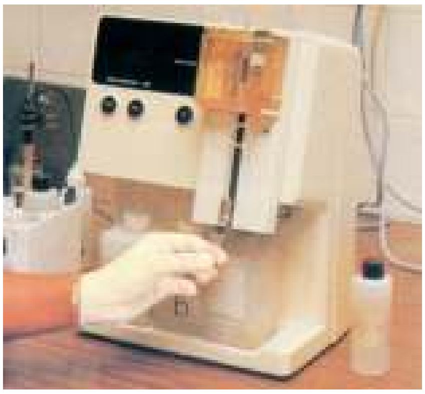


Fig.19.37: Introduction of serum in the electrolyte analyzer

FLAME PHOTOMETRY

BAQ: What is flame photometry? What is the principle of flame photometry and basic working procedure?

Ans: In flame photometry, a solution containing the specimen (serum, urine, etc) is passed under carefully controlled conditions as a very fine spray into the air supply of a burner. In the flame, the solution evaporates, and the substance is first converted to the atomic state or to its constituent radicals, in which the electrons in the outermost shell are in their lowest energy state closest to the nucleus (the ground state). As the temperature rises, the thermal energy of the flame excites these electrons so that they can absorb one or more quanta of thermal energy and move into higher energy orbits further from the nucleus. The electrons in the higher energy orbits are in a metastable state and are prone to return to lower energy orbits, including to the ground state. In doing so, the energy previously absorbed is released as quanta of light. The line spectra are characteristic for each element such as sodium, potassium, lithium, etc. Sodium produces a yellow, potassium a violet, and lithium a red color in the flame.

Principle of flame-photometry: Under constant and controlled conditions, the light intensity of the characteristic wavelength produced by each of the atoms in serum (or urine) is directly proportional to the number of atoms that emit energy, which is directly proportional to the number of atoms present in the sample.

Observe the working of a flame photometer (Figs 19.38–19.40):

Basic steps for the determination of serum sodium and potassium

1. Preparation of working standard: Sodium/potassium: 140/4.0 mEq/L
2. Preparation of dilution of serum
3. Introduce diluted standard to flame photometer and adjust readings: 140/4.0 mEq/L
4. Introduce dilute serum to flame photometer and note the readings.

BAQ: Explain the working of a gas analyzer to determine partial pressures of blood gases: pCO_2, pO_2, blood pH, and serum bicarbonate.

Fig.19.38: Introduction of sodium/potassium standard 140/5 mEq/L

Fig. 19.39: Adjusting standard readings (sodium: 140/Potassium 4)

Fig. 19.40: Display of serum sodium/potassium

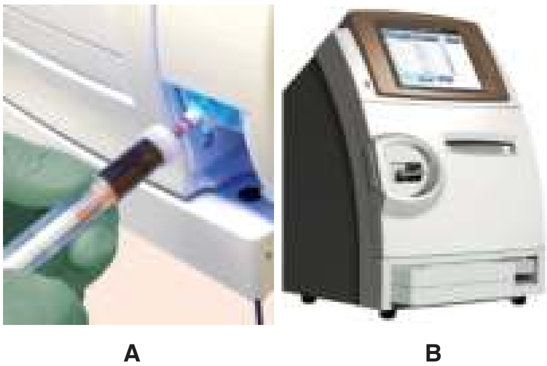


Fig. 19.41: (A) Introduction of heparinized arterial blood to the blood gas analyzer (BGA), (B) Blood gas analyzer

Ans: Procedure:

Introduce heparinized blood in the blood gas analyzer (Fig. 19.41).

Observation: Display the following report in a few minutes:

- Blood pH: 7.28
- PCO_2: 65 mm Hg
- PO_2: 70 mm Hg
- Serum bicarbonate: 29 mmol/L

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

LAQ: What is ELISA? State the principle of ELISA, and mention the types, procedures, and applications.

Ans: Enzyme-linked Immunosorbent Assay (ELISA): Enzyme-linked means any substance like antigen or antibody linked with a specific enzyme covalently and immunosorbent means relating to the use of a substrate specific for the enzyme and consisting of a specific antibody or antigen that chemically combines and absorbs in an insoluble substance like cellulose to select and remove the corresponding specific antigen or antibody from a specimen like a serum.

ELISA types: There are two main types of performing ELISA:

A. Double antibody technique (Sandwich) to detect antigen.

B. Indirect technique (to detect antibodies).

A. Double antibody ELISA (Sandwich) technique (Fig. 19.42)

Standard operation procedure (SOP):

1. On the surface of a test tube (or microtitration plate), a specific antibody is coated.
2. Specimen is added. After mixing, it is kept for incubation (antibody captures antigen).
3. The test tube or well is washed.
4. Enzyme-specific antibody is added and kept for incubation.
5. Enzyme-labeled antibody combines with the antigen.
6. The well is washed, and a specific substrate is added.
7. The enzyme acts on the substrate, and at the end of the reaction, a colored complex is formed.

   If the enzyme is alkaline phosphatase, the substrate is P-nitrophenyl phosphate (PNP)

$$\underset{\text{(Colorless)}}{\text{PNP}} \xrightarrow{\text{alkaline phosphatase}} \underset{\text{(Yellow color)}}{\text{P-nitrophenol}}$$

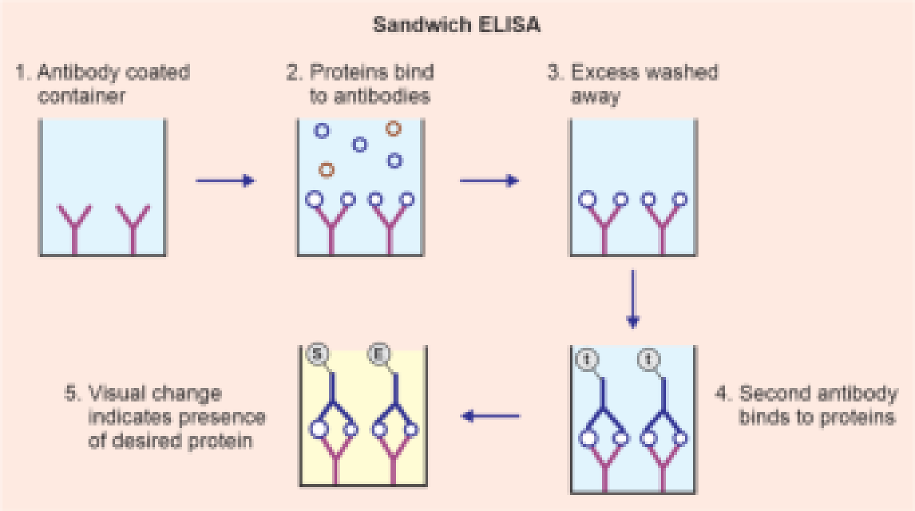

Sandwich ELISA
1. Antibody coated container
2. Proteins bind to antibodies
3. Excess washed away
4. Second antibody binds to proteins
5. Visual change indicates presence of desired protein

Fig. 19.42: Double antibody (sandwich) technique

8. The amount of attached antibody and, therefore, the antigen can be estimated by measuring the color produced using a microplate reader (Fig. 19.43).

B. Indirect ELISA technique (Fig. 19.44)

Standard operation procedure (SOP):

1. A known antigen is attached to the inside surface of a test tube or well.
2. Patient's serum is added and incubated.
3. After washing, an enzyme-labeled antihuman globulin is added. It reacts with the antibody (that has attached to the antigen).
4. The uncombined labeled enzyme is washed from the well (or test tube).
5. A specific substrate is added.
6. Color produced is proportional to the concentration of antibodies in the serum
7. Color is measured using a microplate reader (Fig. 19.44).

ELISA plate readers are designed with various types of microtiter plates and strip formats, providing high standards of accuracy and precision. Filters in the range of 400–700 nm are generally used for the test readings.

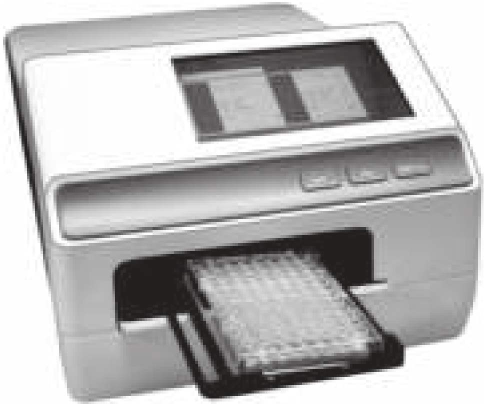


Fig. 19.43: Microplate reader

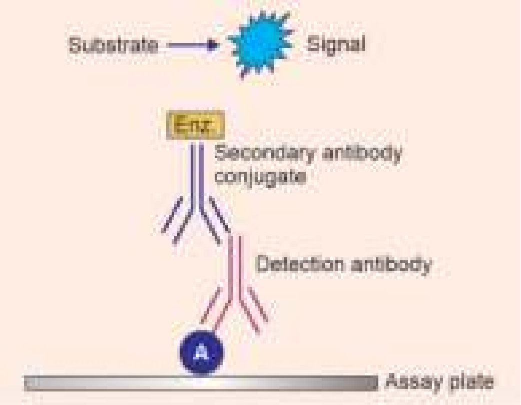

Substrate
Signal
Enz.
Secondary antibody conjugate
Detection antibody
A
Assay plate

Fig. 19.44: Indirect ELISA

Application of ELISA tests:

1. ELISA tests are used for the diagnosis of viral and bacterial diseases by determining antibodies produced by these microorganisms.
2. ELISA tests are used for the identification of IgM (primary antibody) and IgG (secondary antibody) which give an idea about the progression of a specific infectious disease.

IMMUNOTURBIDIMETRY

SAQ: What is immunoturbidimetry? What are the uses of immunoturbidimetry techniques?

Ans: Turbidity produced by antigen–antibody reactions causes a decrease in the intensity of the incident beam of light as it passes through a solution of particles. Immunoturbidity involves the measurement of the decrease in intensity of the incident light beam that is caused by scattering, reflectance, and absorption of the light. A photometer or a spectrophotometer can be used to measure turbidity produced by antigen–antibody complexes.

Uses: Immunoturbidimetry techniques are used for the determination of small concentrations of various proteins such as antigens and antibodies in serum.

IMMUNODIFFUSION

BAQ: What is the immunodiffusion technique? Give an account of the uses of the immunodiffusion technique.

Ans: Immunodiffusion techniques utilize the secondary phase of the antigen–antibody reaction in which an immune precipitate is formed as antigen–antibody complexes cross-link and precipitate. In immunodiffusion, one or both reactants diffuse passively toward each other, or it can be an active diffusion under the influence of an electrical field through the gel matrix. Many qualitative and quantitative immunochemical methods are performed in a semisolid medium such as agar or agarose (Fig. 19.45).

Uses: Immunodiffusion techniques are useful in the identification of various antigens and antibodies in serum.

RADIOASSAYS (RIA)

LAQ: What are radioimmunoassays (RIA)? What is the principle of radioimmunoassays? What are the uses of radioimmunoassays?

Ans: Antigen or antibody can be tagged using radioactive iodine, ^{125}I. In the biochemistry laboratory for the determination of

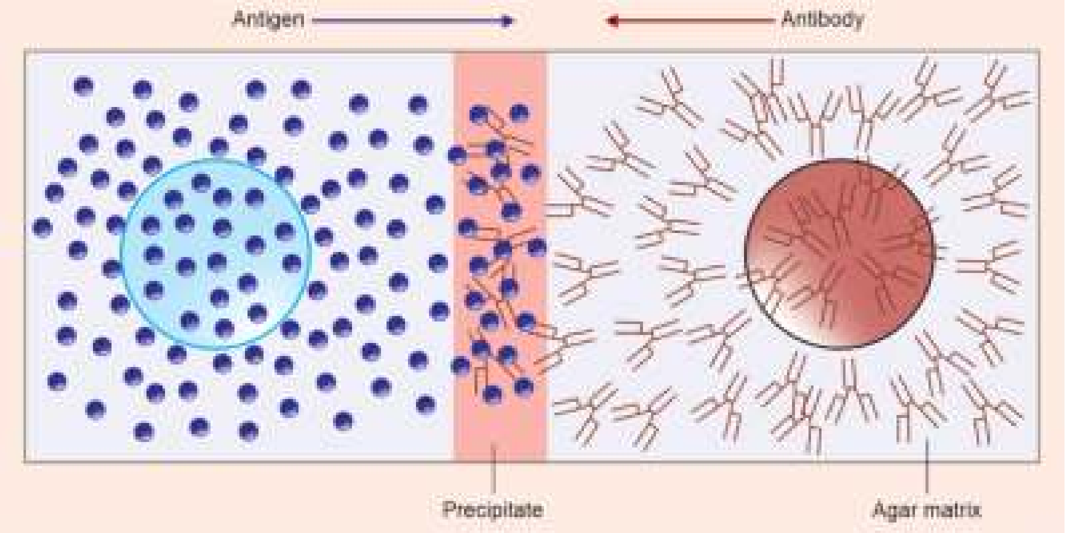


Fig.19.45: Immunodiffusion technique

hormones, a method similar to indirect ELISA can be used. The only difference is, instead of an enzyme-labeled antigen, the radioactive iodine-labeled antigen is used. Radioactive iodine-labeled antigen binds specifically with the specific antibody coated in the test tube. After washing the unbound fraction is removed and the bound fraction containing the antigen is determined by scintillation counters such as gamma counter, which measures gamma rays emitted by the radioactive iodine tag. Thus, the concentration of antigen (e.g. thyroxine) in the serum is determined. A sodium iodide crystal activated with 1% thallium is used as the solid scintillator. The crystal is in the form of a well (Fig. 19.46).

Principle

Example: Determination of thyroxine: T_4

The RIA technique is based on the competition between the unlabeled antigen (the component in the sample, e.g. thyroxine) and a finite amount of the corresponding radiolabeled antigen (^{125}I-labeled thyroxine) added from the reagent kit, for a limited number of antibody binding sites. By specific washing techniques, the unbound fraction of the antigen is removed, and the concentration of the unknown antigen (T_4 in serum) is determined by determining ^{125}I-labeled antigen bound to the antibody by using a γ-counter (Fig.19.46B).

Q: Demonstrate application of radioimmunoassay in the determination of T_4.

1. Specific T_4 antibody-coated tube is taken
2. Serum is added and incubated for the specified time
3. After washing, radioactive iodine-labeled T_4 is added
4. After incubation, washing is performed, to discard unbound fraction
5. Tube is placed on the gamma counter for reading (Fig. 19.46 B)
6. Reading is displayed on the meter of the gamma counter.

Use: For the determination of hormones such as T_3 and T_4 radioimmunoassays can be used.

Q: What is a gamma counter? What is the basic working procedure of a γ-counter?

Ans: The gamma counter is the equipment used to measure the intensity of γ-rays emitted by a radioactive substance like ^{125}I.

Basic working procedure: Gamma rays are emitted from a radioactive tag in the specimen in a test tube when placed on the scintillation counter, γ-rays fall on the sodium iodide crystal, following chemical events take place (Fig. 19.46A):

1. The sodium iodide crystal well is sealed onto a photomultiplier tube to detect light pulses emitted by the crystal at 410 nm.
2. The highly penetrating γ-rays from the radioactive label in the test tube strike the sodium iodide crystal, and photons are released.
3. The photons are detected by the photomultiplier tube, which amplifies the signal to produce a cascade of electrons.
4. The output from the photomultiplier tube can be fed to a preamplifier.
5. The amplified pulse is recorded by a recording device (pulse height analyzer).
6. A scaler counts the number of pulses that arrive in a defined time interval.
7. The output from the scaler can be processed by a microprocessor to display data in chart form and perform calculations to yield quantitative results on the analytes in the sample.

Scintillation Counters

BAQ: Write a brief note on a scintillator.

Ans: The photons emitted by the excited atoms in the detector are picked up by a photomultiplier and thereby converted into an electrical pulse; the size of which is proportional to the number of photons. The scintillator is a clear crystal of sodium iodide containing a trace of thallium iodide (Fig. 19.46A). Thallium-activated sodium iodide crystals are of high density (3.7 g/ml),

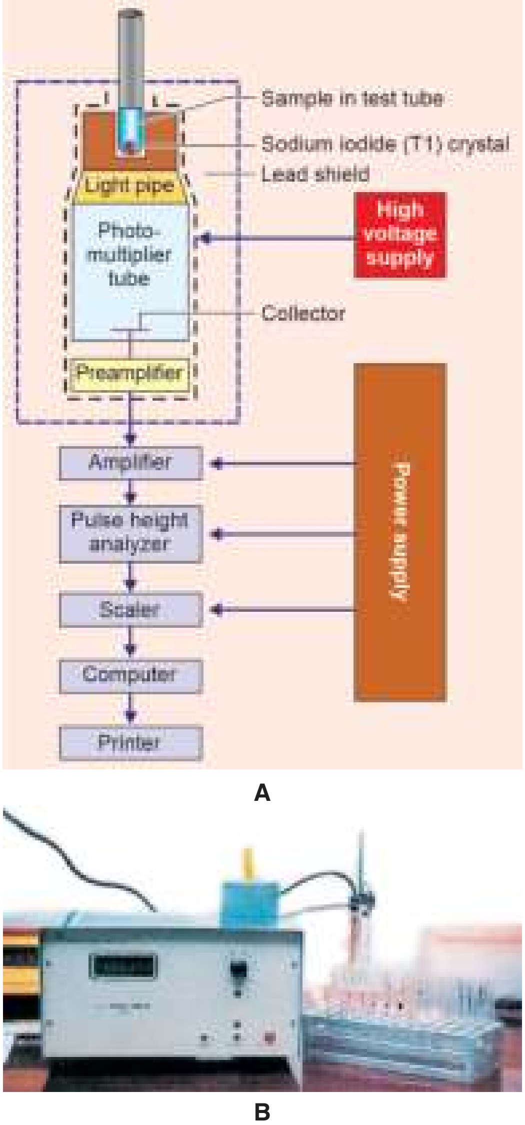


Fig. 19.46: (A) Line diagram of working of gamma counter; (B) Gamma counter

and as iodine has a high atomic number (z = 53), γ-radiation is detected by its photoelectric effect. The thallium atoms in the crystal lattice alter the electron energy bands locally. When these electrons are excited, they emit the scintillations on returning to a lower level. As the crystal is hygroscopic, it is enclosed in a watertight cover.

BAQ: What is the use of radioimmunoassays in the diagnosis of infectious diseases?

Ans: Radioimmunoassay (RIA) is used for the determination of specific antigens or antibodies in the specimen, such as serum or CSF. Methods based on the RIA technique are highly specific and very sensitive. However, these methods require a piece of separate equipment like a gamma counter to measure radioactivity. In the RIA, the radioactivity of a specific isotope labeled antibody or antigen is used to detect and for the quantitative determination of antibody or antigen in the specimen.

POLYMERASE CHAIN REACTION (PCR)

LAQ: What is polymerase chain reaction (PCR)? State the principle of PCR. Describe the procedure of PCR.

Ans: The polymerase chain reaction (PCR) means the preparation of millions of copies of a specific DNA. PCR provides an efficient method to obtain a sufficient quantity of target DNA required for the identification of specific DNA related to any person, specimen, or organism.

Principle

PCR technology generates sufficient quantities of DNA by increasing (amplifying) the number of copies of the target region of DNA (the template, which serves as the pattern for making copies) without altering the template nucleotide sequence. At the end of a PCR experiment, over a million copies of the original DNA of interest can be obtained as complementary or copy DNA (cDNA).

Requirements of PCR

1. The double-stranded DNA (dsDNA) of interest separated from the sample. The various samples used for PCR are blood, serum, semen (for forensic purposes), cells grown in culture (for research), and tissue biopsy (from either formalin—fixed paraffin—embedded tissues or frozen tissue sections).
2. The primers: Two primers, known as antisense and sense primers, are required.

A primer is typically a sequence in the range of 15–25 nucleotides whose sequence is complementary to the known nucleotide sequence of interest. Each primer sequence is specific for each unique gene sequence.

3. Thermus aquaticus (Taq): Since PCR is performed at high temperatures (55°–94°C), eucaryotic DNA polymerase that functions at 37°C would be degraded. Thermus aquaticus (Taq), which thrives at an optimal temperature of 90°C in hot springs, provides the main source of thermostable DNA. DNA polymerase is cloned from *Pyrococcus furiosus* (Pfu), an organism that grows optimally in geothermal marine sediments at 100°C.
4. The buffer systems and dNTPs: The optimal PCR conditions are significantly influenced by the $MgCl_2$ concentration of the buffer system. The four dNTPs are added to the reaction mixture at a concentration of 50–200 nanomoles to provide the source of nucleotides used to extend the primer in the reaction catalyzed by the thermostable DNA polymerase.

Standard operation procedure (SOP)

1. DNA extraction from a specific source.
   - Sample is mixed with saline–EDTA solution (EDTA chelates metal ions needed for DNase and inhibits its activity)
   - Lysozyme and sodium dodecyl sulfate (SDS) are added to rupture the cells in the sample.
   - The mixture (sample + EDTA) is kept with lysozyme at 37°C and afterwards at 60°C with SDS.
   - After cooling to room temperature, with a high salt concentration (0.15 M NaCl) which prevents DNA strand separation, sodium chlorate solution is added, which denatures the proteins.
   - DNA extraction is carried out by organic solvent mixture (chloroform, isoamyl alcohol mixture) followed by centrifugation at 10,000 g.
   - DNA strands are separated and dissolved in concentrated saline citrate and treated with RNAse to remove contaminated RNA fractions (at 37°C). After centrifugation, the upper layer is used for PCR methodology.
2. Denaturation of DNA (Fig. 19.47)
   - DNA denaturation (often called melting) is achieved by heating dsDNA at 94°C for 1 minute.
3. Annealing the primers to the DNA (Fig. 19.47)
   - Annealing with the primer takes place at 60°C for 2 minutes.
4. Extending the primers (Fig. 19.47).
   - Primer extension is performed at 72°C for 2 minutes. When the primer extension is terminated, one cycle of PCR is completed by doubling the number of dsDNA molecules.
   - Each cycle of PCR follows sequentially, utilizing the extended primers from the previous cycle as templates. This creates a chain reaction.
   - About 20 cycles of PCR (2^{20}) are required to produce a million-fold amplification of ds DNA extracted from the sample of choice.

   Denaturation, annealing, and extension procedures are carried out by a thermal cycler (PCR equipment).
5. Identification of DNA: DNA identification is done by DNA electrophoresis on agarose gel and then by autoradiography technique (Fig. 19.51). DNA is first treated with thymidine bromide. DNA stained with thymidine bromide can be analyzed using an electronic UV transilluminator.

Q: Write a note on the applications of PCR.

Ans: The following are the various applications of PCR technology:

1. PCR can identify organisms that cause infectious diseases such as COVID-19, dengue fever, diphtheria, polio, etc.
2. Tissue biopsy specimens can be analyzed by PCR for gene mutations that signify the

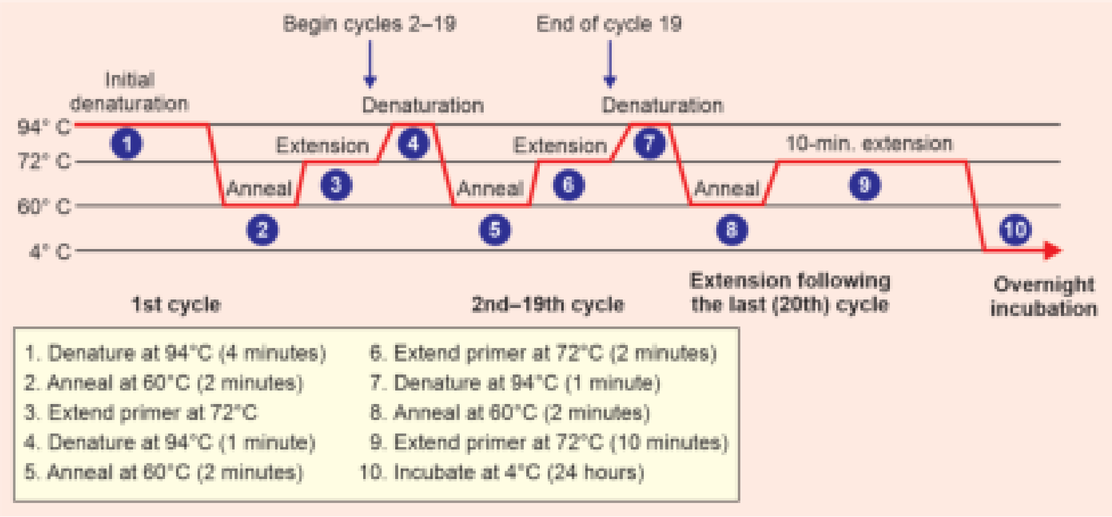
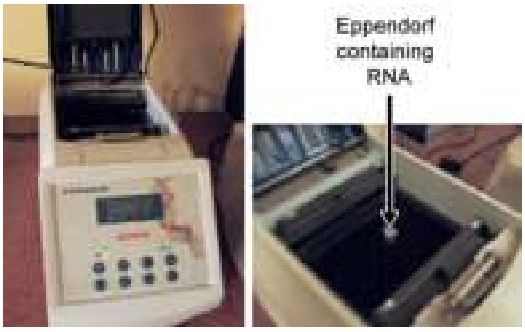
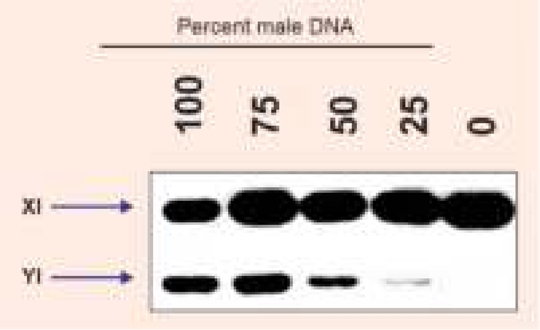

Begin cycles 2–19
End of cycle 19
Initial denaturation
Denaturation
Denaturation
94° C
72° C
60° C
4° C
Extension
Extension
10-min. extension
Anneal
Anneal
Anneal
1
2
3
4
5
6
7
8
9
10
1st cycle
2nd–19th cycle
Extension following the last (20th) cycle
Overnight incubation
1. Denature at 94°C (4 minutes)
2. Anneal at 60°C (2 minutes)
3. Extend primer at 72°C
4. Denature at 94°C (1 minute)
5. Anneal at 60°C (2 minutes)
6. Extend primer at 72°C (2 minutes)
7. Denature at 94°C (1 minute)
8. Anneal at 60°C (2 minutes)
9. Extend primer at 72°C (10 minutes)
10. Incubate at 4°C (24 hours)

Fig. 19.47: Basic procedure steps of PCR


Eppendorf containing RNA

Fig, 19.48: Thermal cycler (PCR equipment)


Percent male DNA
100
75
50
25
0
XI
YI

19.50: Separated DNAs, identification of DNA by autoradiography

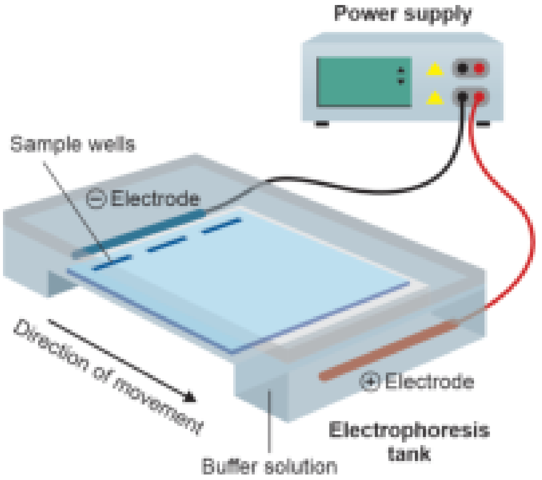

Power supply
Sample wells
⊖ Electrode
Direction of movement
⊕ Electrode
Electrophoresis tank
Buffer solution

Fig. 19.49: DNA electrophoresis (RHS: Power pack, LHS: Buffer tank with gels)

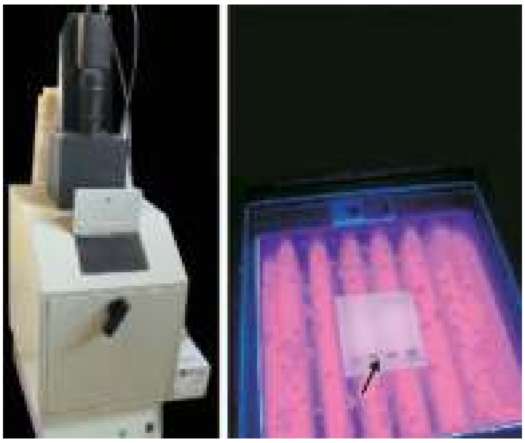


Fig. 19.51: Use of UV-transillluminator (LHS), display of illumination (RHS)

early development of cancer. The mutations of genes that control cell growth, tumor suppression, and apoptosis (programmed cell death) are strongly associated with the molecular pathogenesis of carcinogenesis. These mutations may precede dysplasia, which is a cellular change that is often a precursor to cancer.

3. The DNA of viruses associated with specific cancers can also be identified by PCR. For example, cervical scrapping can be tested to see if they contain intact DNA from the human papillomavirus (HPV), a causative agent of cervical cancer. By using PCR to detect HPV DNA, the early detection of cervical carcinoma is possible.
4. PCR can also detect genetic mutations associated with other diseases. For example, coronary artery disease (CAD) due to atherosclerosis is strongly associated with mutations of the gene that encodes for low-density lipoprotein receptor (LDL-R). This mutation results in dysfunctional LDL-R contributing to increased blood LDL levels and other clinical signs of an atherosclerotic process. If dysfunctional LDL-R is detected before the clinical signs of atherosclerosis, early treatment with diet and exercise may reduce the risk of plaque development and hence CAD.
5. In the case of breast cancer, early detection of mutated genes can precede the histopathologic observation of malignant cells in a breast mass biopsy.
6. Insulin-dependent diabetes mellitus (IDDM) has also been associated with genetic mutations, which may precede clinical symptoms. Hence an individual with documented gene mutations for IDDM may be a candidate for early nutritional support, insulin administration, and an exercise program to regulate blood glucose as soon as possible.
7. Mitochondrial DNA extracted from saliva residue on stamps, shed hair, bone, platelets, and leukocytes have been useful in both diagnostic and forensic pathology.

PCR Equipment (Thermal cycler) (Fig. 19.48)

SAQ: Write a brief note on the thermal cycler, used in radioimmunoassays.

Ans: PCR equipment consists of a thermal cycler to program the temperature and time parameters for each of the three steps for PCR. The thermal cycler contains a heating block with the necessary software for the investigator to set the appropriate temperature (94°, 60°, and 72°) and times in specific minutes. The heating block holds the microcentrifuge tubes that contain the PCR mixture.

BAQ: How length of cDNA is determined?

Ans: Determination of length of cDNA

An aliquot of the cDNA is analyzed by agarose gel electrophoresis to determine the bp length (bp length is equal to the number of nucleotides in the cDNA product). Commercially prepared DNA size markers are run in parallel with the PCR aliquot. The electrophoretically separated pattern is stained with the fluorescent dye ethidium bromide. The fluorescence can be photographed by using a Polaroid or digital camera. The bp length of the PCR product can be measured by comparing the PCR signal on the gel to that of the closest DNA size marker with which it co-migrates in electrophoresis (Fig. 19.49).

BAQ: What is real-time PCR (RT-PCR) and its importance?

Ans: In real-time PCR the target amplification and detection steps occur simultaneously in the same tube. Real-time PCR requires special thermal cyclers with precision optics that can monitor the fluorescence emission from the sample wells. The computer software monitors the data generated throughout the PCR at every cycle and generates an amplification plot for each reaction (Fig. 19.52).

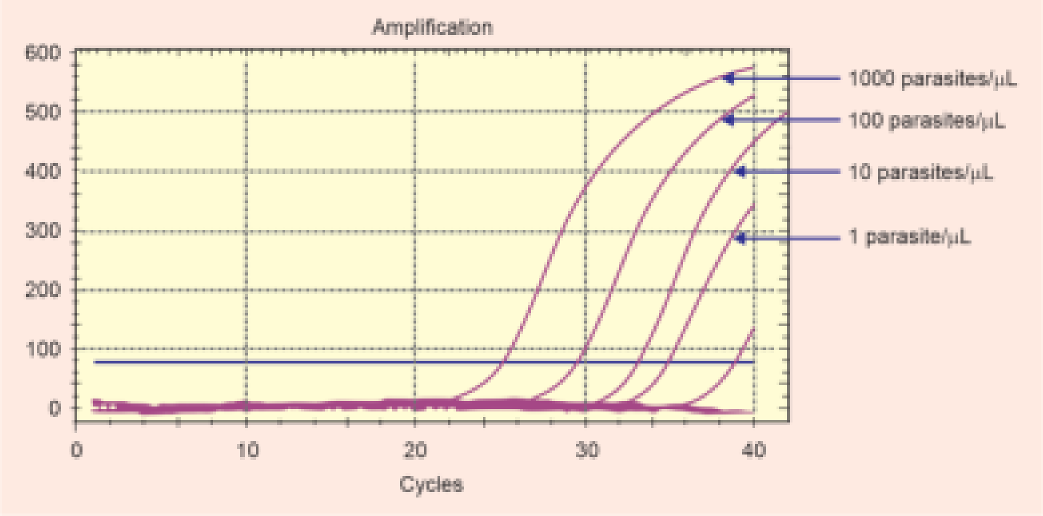


Fig. 19.52: RT-PCR amplification cycles

Importance: The RT-PCR test is extremely useful in the diagnosis of viral and bacterial diseases, by determining their respective RNAs and DNAs. Examples: Diagnosis of AIDS, COVID-19, viral hepatitis, etc.

Competency achievement: The student should be able to:

SU9.1: Choose appropriate biochemical, microbiological, pathological, and imaging investigations and interpret the investigative data in a surgical patient

SAQ: Enumerate uses of radioisotopes in medicine.

Ans: The various uses of radioisotopes (and radiotherapy) are as follows:

1. To find out the functions of specific organs of a person
2. To treat clinical conditions, especially cancer, using radiation to destroy specific targeted cells.
3. For the sterilization of medical equipment

SAQ: Write two names of isotopes used in medicine.

1. Radioactive iodine (^{125}I) is used in radioimmunoassays.
2. Technetium-99 (Tc-99) is used in positron emission tomography (PET)

SAQ: What is a CT scan?

Ans: CT scan is a computerized X-ray imaging scan procedure in which a narrow beam of X-rays is directed at a patient and these rays are then rotated around the body. The signals produced are processed by a computer of the machine, which generates cross-sectional images (slices) called tomographic images. Once a sufficient number of successive slices are collected by the computer, these are digitally "stacked" together to form a three-dimensional (3D) image of the patient. CT scan allows easier identification of the basic structure of a scanned region of a patient's body.

SAQ: What is MRI?

Ans: MRI means magnetic resonance imaging. It is a medical imaging technique that uses a magnetic field and computer-generated radio waves to create detailed images of the organs and tissues of a patient's body.

SAQ: What is a PET scan?

Ans: PET means positron emission tomography. The PET scan is an imaging test that uses radioactive material such as Technetium-99 (Tc-99) to diagnose various diseases related to various organs such as the brain, heart, liver, kidneys, etc.

SAQ: What is nuclear medicine?

Ans: In nuclear medicine, radiations are used to get diagnostic information using radioisotopes. This type of therapy is known as radiotherapy. Various organs such as the liver, pancreas, thyroid gland, bones, heart, and many other organs can be easily imaged to diagnose disorders in their structures and functions. The most common radioisotope used in diagnosis is technetium-99 (Tc-99).

BAQ: Describe the importance of PET scans briefly.

Ans: A radioactive dose is given to the patient by injection or orally and the activity of the radioactive substance (tracer) in the organ is then studied either as a two-dimensional picture, or as a three-dimensional picture (tomography).

The radioactive tracers emit gamma rays from within the body. These tracers are generally short-lived isotopes linked to chemical compounds that facilitate specific physiological processes to be scrutinized.

Positron emission tomography (PET) is a precise and sophisticated technique that uses a positron-emitting radionuclide and a cyclotron. A cyclotron is a type of particle accelerator that repeatedly propels a beam of charged particles (protons) in a circular path. As the positron-emitting nucleotide decays, it emits a positron, which promptly combines with a nearby electron resulting in the simultaneous emission of two identifiable gamma rays in opposite directions. These are detected by a PET camera and give very precise indications of their origin.

Clinical applications

The PET scan is performed on any part of the body or on the entire body to detect any specific disordered structure, biochemical and physiological events. The PET scan is a combination of nuclear medicine and biochemical analysis. A PET scan is useful to visualize the biochemical changes taking place in the body during the various types of metabolism reactions.

The most important clinical role of PET scans is in oncology. Using fluorine-18 as a tracer it has proven to be the most accurate non-invasive method of detecting and evaluating most cancers. The PET scan is also used in cardiac and brain imaging.

SAQ: What is the difference between a CT scan, MRI, and PET scan?

Ans: Computed tomography (CT) and magnetic resonance imaging (MRI), show anatomic detail. PET images show biochemical and physiological events. PET offers substantial advantages over anatomic imaging methods in oncologic imaging. PET can better distinguish between benign and malignant lesions compared to CT and MRI scans.

Chapter

20

Medical Biochemistry Practicals

Viva voce questions are included in all the laboratory experiments and the standard operation procedures (SOPs) of the experiments.

Instruction for students: For writing journals use the format of Experiment No. 18

PRIMARY STANDARDS, CALIBRATORS AND QC SERUM

SAQ: What are primary standards, calibrators, and quality control (QC) serum?

Ans:

1. Primary standards are solutions of known concentration, dissolved in a suitable solvent. Example: 100 mg/dl glucose standard is prepared in saturated benzoic acid.
2. Calibrators are solutions of known concentration. Example: Bilirubin standard 10 mg/dl is prepared using cobalt nitrate. Color of this solution = OD of 10 mg bilirubin.
3. Quality control (QC) serum: This serum contains all the constituents of serum such as glucose, urea, SGPT, SGOT, calcium, proteins, etc. of known concentration. QC serum can be used as a primary standard.

DIAGNOSTIC KITS

LAQ: What are diagnostic kits? What are the advantages of using a diagnostic kit?

Ans: There has been tremendous development in techniques and methodologies in clinical biochemistry. Diagnostic kits have been extensively used by clinical laboratories to keep pace with the latest developments in clinical practice. A diagnostic kit contains specific reagents (and other accessories) for testing specimens.

Advantages of using a diagnostic kit (Fig. 20.1)

1. It is not necessary to prepare various reagents required for a test.
2. A standardized set of reagents is readily available with appropriate standards and calibrators.

Criteria for the Selection and Use of a Diagnostic Kit

Q: What criteria are used for the selection and use of a diagnostic kit?

Ans: The following criteria are used for the selection and use of a diagnostic kit:

A. It is necessary to check product information by referring to the label and product insert.
B. Following information (based on WHO criteria) must be provided by the manufacturer:

Labeling and product information: The container for each reagent should provide the following information clearly:

1. The name of the test should be adequate to identify the product and its use.

2. **Intended use:** The nature of the product and its intended use must be made very clear.
3. **Quantity:** The container label of each component should give the volume of the contents.
4. **Composition:** The composition and concentration of the reagent should be listed on the label. Other information, i.e. principle of the test, reference range, procedure, precautions, etc. should be included in the package insert.
5. Lot number should be printed on the container and the package label.
6. **Storage:** Any critical conditions of storage (at room temperature, i.e. 25°C ± 5°C, 2–8°C or 0–4°C) to ensure the stability of the product should appear on both labels and product insert.
7. Expiry date applicable to recommended storage condition should appear on both labels.
8. Precautions in handling the product should be displayed prominently by using appropriate safety signs such as poisonous, corrosive, toxic, radioactive, etc.
9. Name and address of the manufacturer (and supplier) should be printed on the labels.
10. The insert of the product should include the following information regarding a test:
    - Name of the test
    - Clinical significance
    - Type of specimen
    - Reference ranges (for male, female age groups, etc.)
    - Requirements (glassware, plasticware, type of reagent grade water, equipment, and instruments)
    - Reconstitution of reagents and storage thereafter.
    - Stability of reagents
    - Test procedure
    - Calculations of test resultant test value
    - Sources of error
    - Procedure limitations
    - Quality control

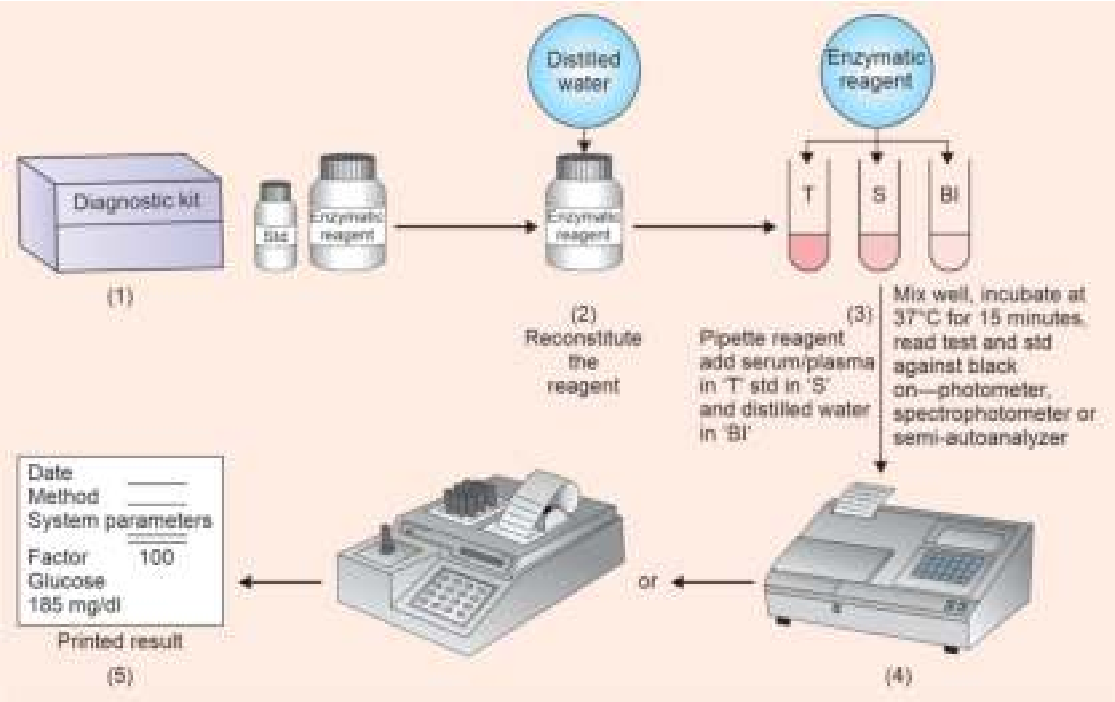

Distilled water
Enzymatic reagent
Diagnostic kit
Std
Enzymatic reagent
Enzymatic reagent
T
S
Bl
(1)
(2)
Reconstitute the reagent
(3)
Pipette reagent add serum/plasma in 'T' std in 'S' and distilled water in 'Bl'
Mix well, incubate at 37°C for 15 minutes, read test and std against black on—photometer, spectrophotometer or semi-autoanalyzer
Date
Method
System parameters
Factor 100
Glucose
185 mg/dl
Printed result
(5)
or
(4)

Fig. 20.1: Use of a diagnostic kit and standard operation procedure for the determination of glucose, uric acid, cholesterol, triglycerides, etc.

Competency achievement: The student should be able to:

BI 11.21: Demonstrate estimation of glucose

Competency achievement: The student should be able to:

BI 11.17: Explain the basis and rationale of biochemistry tests done in diabetes mellitus

Experiment 1: Determination of plasma glucose by glucose oxidase method.

Q: What are the instructions given to the patient for fasting blood glucose test?

Ans: *Patient care preparation:* The patient should be fasting for at least 8 hours.

Q: What type of blood is used for blood glucose tests? How much blood is collected

Ans: *Specimen:* 2 ml of blood is collected in a fluoride tube or a bulb. Fluoride plasma is used for the test. Neonatal blood should be drawn from the heel stick.

Q: What are the normal ranges (reference ranges) of plasma fasting glucose?

Ans: Interpretative reference ranges

- Premature infants : 40–65 mg/dl
- 0–2 years : 60–110 mg/dl
- 2 years to adult : 60–115 mg/dl
- Adults : 70–110 mg/dl

Q: What are the panic ranges of plasma fasting glucose?

Ans: Possible panic ranges

- Adult (male and female) : >400 mg/dl
- Adult (male) : <50 mg/dl
- Adult (female) : <40 mg/dl
- Infants : <40 mg/dl

Q: What is post-prandial plasma glucose?

Ans: Post-prandial plasma glucose means, plasma glucose 2 hours after normal food intake (lunch).

Q: What is post-glucose plasma glucose?

Ans: Post-glucose plasma glucose means, plasma glucose 2 hours following intake of 75 g glucose.

Q: What is the clinical significance of a fasting blood glucose test?

Ans: Determination of fasting plasma glucose is important to find out if a person is suffering from hyperglycemia due to a deficiency of insulin in diabetes mellitus. This test is also important to find out if a patient is suffering from hypoglycemia, due to hypersecretion of insulin.

Determination of fasting plasma glucose is also important in hypo- and hyper-secretions of thyroxine, epinephrin, glucagon, and growth hormones.

Q: What are the other causes of high plasma glucose?

Ans: The other causes of high plasma glucose include:

- Recent or current IV infusions of glucose.
- Stress state such as myocardial infarction, brain damage, convulsive episodes, trauma, general anesthesia, etc.
- Cushing's disease, acromegaly, pheochromocytoma, severe liver disease, and pancreatitis.
- The drugs, such as thiazide and other diuretics, corticoids, etc. are reported to cause high plasma (or serum) glucose levels.

Q: What are the various causes of hypoglycemia?

Ans: Fasting hypoglycemia is likely to be observed in:

- Pancreatic islet cell tumors
- Addison's disease
- Hypopituitarism
- Effects of hypoglycemic drugs such as insulin and oral hypoglycemic drugs
- Fulminant hepatic necrosis
- Alcoholism with starvation
- Malabsorption

Post-prandial hypoglycemia may occur after:

- Gastrointestinal surgery
- Hereditary fructose intolerance and galactosemia.

Determination of Post-prandial (PP) Plasma Glucose

Q: What are the instructions given to the patient for the plasma glucose PP test?

Ans: Patient care, preparation

- Patient is allowed his/her usual meal (adequate breakfast or lunch). The patient must complete the meal within 15–20 minutes.
- Specimen is collected 2 hours from the beginning of a meal.

Q: What are the normal ranges (reference ranges) of plasma glucose PP?

Ans: Interpretative reference range

- In an unstressed adult male (not receiving any medication) <140 mg/dl.
- In an unstressed non-pregnant woman (not receiving any medication) <140 mg/dl
- 140–200 mg/dl is classified as impaired glucose tolerance.
- A 2-hour result >200 mg/dl, on at least two occasions, supports the diagnosis of diabetes mellitus.

Q: What are the instructions given to the patient for random plasma glucose tests?

Ans: Patient care preparation

- No specific preparation; specimens are collected anytime within 24 hours.

Interpretative reference range

- Adult : 70–150 mg/dl

Plasma glucose of >200 mg/dl in a non-stressed, ambulatory subject supports the diagnosis of diabetes mellitus.

Q: What are the possible panic ranges of random plasma glucose?

- Adult (male and female) : >400 mg/dl
- Adult (male) : <50 mg/dl
- Adult (female) : <40 mg/dl
- Neonates : <40 mg/dl

Q: What is the clinical significance of random plasma glucose test?

Ans: Clinical significance of random plasma glucose test:

- Random plasma (or serum) glucose test is mainly performed on patients hospitalized due to various reasons and also before giving anesthesia.
- This test may be important in diabetic patients under insulin or oral hypoglycemic drug therapy who show symptoms of hypoglycemia.
- In pregnant women, a value >105 mg/dl usually prompts further investigation.

Q: How blood specimen is collected for the post-glucose (PG) determination of plasma glucose?

Ans: Patient care, preparation

- After collecting the fasting specimen, 75 g glucose is given to the patient. (Glucose should dissolve completely in drinking water, and the patient should drink it completely. Little lime juice may be added to the glucose solution to make the drink palatable). Time is noted when the patient consumes the glucose solution completely.
- Exactly after 2 hours, a specimen is collected (in fluoride anticoagulant).

NOTE

1. **Glucose tolerance** means the ability of the body to utilize glucose. Glucose tolerance decreases in diabetes mellitus and certain endocrine disorders like hyperthyroidism, hyperpituitarism and hypoadrenalism. Glucose tolerance increases in the hyper-secretion of insulin by the β cells of the pancreas.
2. **Glucose tolerance test (GTT):** Blood sugar in the case of a normal individual remains fairly constant throughout the day, about 1 mg/ml. Following food intake, there is a temporary rise in blood sugar, the extent, and duration of which depends on the type of food taken. Blood sugar level returns to normal within two to three hours after taking food. In decreased glucose tolerance, however, blood glucose level does not return to normal within 2 to 3 hours after food intake. This effect of ingested carbohydrates can be studied under reasonably standard conditions employing the glucose tolerance test.

Instructions given to the patient for GTT

1. The patient should be on a balanced diet (containing normal daily requirements

of carbohydrates) for at least 2 to 3 days before the test.

2. Patient should report to the laboratory after fasting for 8–12 hours. He can drink water.
3. Patient should bring a fasting midstream urine sample collected in a clean and dry 100 ml bottle.
4. Patients should be in a position to wait at the laboratory for at least 2–3 hours, since five blood samples are collected at the interval of 30 minutes.

Instructions to the technician for GTT

1. First, test the collected fasting urine specimen for glucose. If glucose is present, then do not perform GTT by giving glucose; instead, a post-prandial sample is collected.
2. Collect a fasting blood sample, 2–3 ml in a fluoride bulb. If glucose is absent in the fasting urine, then follow the instructions given below:
    a. Give 75 gm or 100 gm of glucose (1.75 g/kg weight, dissolved in water) to the patient. The addition of lemon juice lessens the risk of the patient vomiting. Note the time.
    b. Collect four more samples at half-hour intervals for two hours after the glucose has been taken.
    c. Four urine samples are collected after the collection of each blood sample. (If the patient is unable to give four urine samples, collect at least two urine samples at the one-hour interval).
    d. Determine blood and urine sugar by the specific method used in the laboratory.
    e. Prepare a glucose tolerance curve by plotting time on X-axis and plasma glucose values on Y-axis (Fig. 20.2).

Q: What are the normal ranges (reference ranges) of plasma glucose PP?

Ans: Interpretative reference range

- Adult (male and female): 70–110 mg/dl
- Infants: 60–110 mg/dl

The following format is for writing the experiment in the journal

Determination of serum/plasma glucose (Fig. 20.1)

Q: What method is used for plasma glucose tests?

Ans: Method

Glucose-oxidase method

Q: What is the principle of plasma glucose, glucose-oxidase method?

Ans: Principle: Glucose oxidase reagent contains two enzymes: Glucose oxidase and peroxidase. The glucose oxidase enzyme in the reagent acts on glucose to give gluconic acid and hydrogen peroxide. The overall reaction is:

$$\text{Glucose} + H_2O + O_2 \rightarrow \text{gluconic acid} + H_2O_2$$

The hydrogen peroxide is broken down into water and oxygen by peroxidase:

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + O_2$$

The oxygen reacts with 4-aminophenazone in the reagent, in the presence of phenol to form a pink-colored compound and intensity (OD) which can be measured at 530 nm (green filter).

Normal values: Serum/plasma glucose (fasting): 70–110 mg/dl, post-prandial (PP): Up to 140 mg/dl.

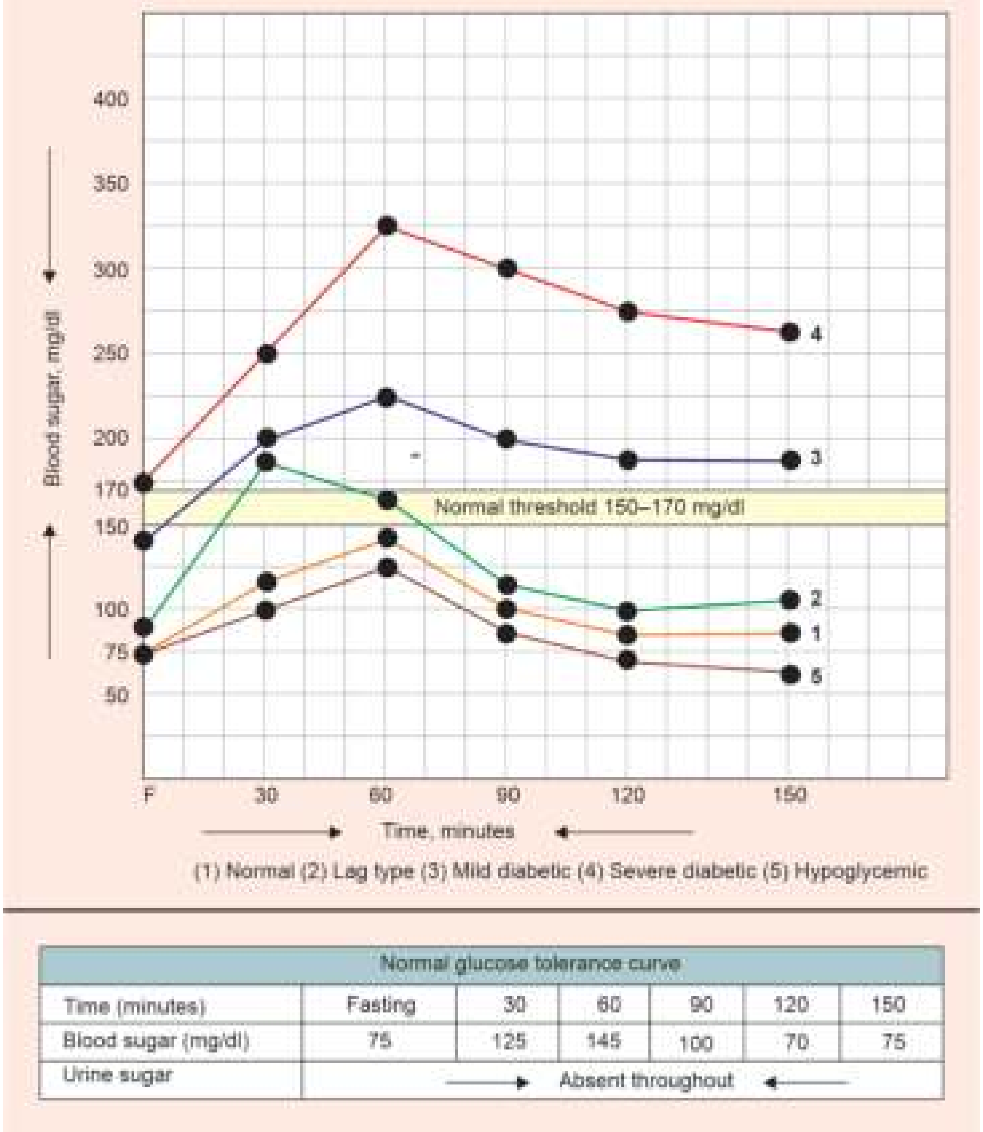
Sample material: Fluoride plasma or serum is collected within 30 minutes of blood collection.

Reagents

1. **Buffer enzymes:** This reagent contains the following constituents in phosphate buffer (M/10, pH 7.0): Glucose oxidase, peroxidase, and 4-aminophenazone with sodium azide as a preservative.
2. **Phenol reagent:** Ready to use
3. **Glucose standard:** 100 mg/dl.

Stability of the reagents

- Reagents 1 and 2 are stable at 2–8°C for 6 months.



Normal glucose tolerance curve						
Time (minutes)	Fasting	30	60	90	120	150
Blood sugar (mg/dl)	75	125	145	100	70	75
Urine sugar	Absent throughout					

Fig. 20.2: Glucose-oxidase method

- Reagent 3 is stable for one year when refrigerated.

Preparation of glucose reagent

Mix 2 parts of buffer/enzymes reagent and 1 part of phenol reagent to get glucose reagent. It is preferable to prepare the reagent fresh daily.

Q: What is the standard operation procedure (SOP) of plasma glucose test by glucose oxidase method?

Ans: Standard operation procedure (SOP): Pipettes in the tubes are labeled as given in Table 20.1.

Table 20.1

		Test	Standard	Blank
1.	Glucose reagent, ml	3.0	3.0	3.0
2.	Plasma/serum, ml	0.02	—	—
3.	Glucose std.: 100 mg/dl. ml	—	0.02	—
4.	Distilled water, ml	—	—	0.02

Mix and keep at 37°C for 15 minutes or at room temperature for 30 minutes. Measure the intensities of the color at 530 nm (green filter).

Calculations

$$\text{Plasma (or serum) glucose, mg / dl} = \frac{\text{OD test}}{\text{OD std.}} \times 100$$

Procedure limitations: The linearity of the method is up to 500 mg/dl. For sample values above 500 mg/dl, dilute the sample 1:2 with distilled water and repeat the assay. Apply the dilution factor to calculate the final results.

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.17: Explain the basis and rationale of biochemistry tests done in renal failure, gout

Competency achievement: The student should be able to:

BI11.21: Demonstrate estimation of serum urea

Experiment 2: Determination of serum (or plasma) urea nitrogen by Berthelot reaction method.

Q: What are the instructions given to the patient for the serum urea nitrogen test?

Ans: Patient care, and preparation: The patient should be fasting for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

NOTE

Serum urea can be determined also in the term of serum urea nitrogen. The following formula is for the conversion of urea to urea nitrogen:

Urea = 2.14 × Urea nitrogen.

Q: What type of blood is used for serum urea nitrogen test? How much blood is collected?

Ans: Specimen: 5–7 ml of blood is collected in a plain tube. The serum is separated from the clotted blood.

Q: What are the normal ranges (reference ranges) of serum urea nitrogen?

Ans: Interpretative reference range

- Birth to 1 year : 4–16 mg/dl
- 1 to 40 years : 7–21 mg/dl
- Gradual slight increase occurs over 40 years of age.
- Possible panic range: BUN > 100 mg/dl

Q: What method is used for serum urea nitrogen?

Ans: Method: Berthelot-reaction method

Q: What is the clinical significance of serum urea nitrogen

Ans: Clinical Significance

- **Elevated levels of urea** are observed in pre-renal, renal, and post-renal conditions.
- **Pre-renal conditions:** Diabetes mellitus, dehydration, cardiac failure, hematemesis, severe burns, high fever, etc.
- **Renal conditions:** Diseases of kidneys.
- **Post-renal conditions:** Enlargements of the prostate, stones in the urinary tract, tumor of the bladder.
- **Decreased values** have been reported in severe liver disease, protein malnutrition and pregnancy.

Q: What is the principle of the serum urea nitrogen test?

Ans: Test principle: The procedure is based on the Berthelot reaction. Urease in the reagent splits urea into ammonia and carbon dioxide. The ammonia reacts with phenol in the presence of hypochlorite to form indophenol, which with alkali, gives a blue-colored compound. The intensity of the colored compound can be measured at 546 nm (green filter).

Sample material: Serum or heparinized plasma

Interfering substances: The method is relatively free of interference from hemoglobin and bilirubin. Anticoagulants such as fluoride and ammonium salts should not be used for blood collection.

Requirements

1. Test tubes: 15 × 125 mm
2. 1.0 ml, 5.0 ml, 0.1 ml graduated pipette

3. Push button pipette (20 µl).
4. Stopwatch
5. Water bath

Reagents

1. Urease/Buffer: pH 7.0 (0.05 M).
2. Phenol reagent
3. Hypochlorite reagent
4. Standard urea nitrogen: 20 mg/dl

Stability of the reagents: All the reagents are stable at 2–8°C for three months.

Q: What is the SOP of the serum urea nitrogen test?

Ans: Standard operation procedure (SPO): Pipettes in the tubes are labeled as given in Table 20.2.

Table 20.2

		Test	Standard	Blank
1.	Urease/buffer reagent. ml	0.5	0.5	0.5
2.	Serum/plasma, ml	0.02	–	–
3.	Standard urea nitrogen: 20 mg/dl. Mix, and keep at 37°C for 10 minutes	–	0.02	–
4.	Phenol reagent, ml	1.0	1.0	1.0
5.	Hypochlorite reagent, ml Mix, and keep at 37°C for 10 minutes.	1.0	1.0	1.0
6.	Distilled water, ml	5.0	5.0	5.0

Mix thoroughly and read optical densities of test and standards against blank at 546 nm (green filter, 520–560 nm).

Calculations

$$\begin{array}{l}\text{Serum (or plasma)}\\ \text{urea nitrogen, mg/dl}\end{array} = \frac{\text{OD test}}{\text{OD std.}} \times 20$$

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.7: Demonstrate estimation of serum creatinine and creatinine clearance

Experiment 3: Determination of serum creatinine by alkaline picrate method

Q: What are the instructions given to the patient for the serum creatinine test?

Ans: Patient care and preparation: The patient should be fasting for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Q: What is the clinical significance of the serum creatinine test?

Ans: Clinical significance: Serum creatinine is increased in renal failure. Increased serum creatinine concentration above 1.5 to 2.0 mg/dl is virtually diagnostic of renal disease. Elevated values are also observed in certain other conditions like congestive heart failure, shock, and mechanical obstruction of the urinary tract.

Q: What method is used for serum creatinine?

Ans: Name of the method: Alkaline-picrate method

Q: What reaction is used for the serum creatinine test?

Ans: Name of the reaction: Jaffe reaction

Q: What is the test principle of the serum creatinine test?

Ans: Test principle: Creatinine reacts with picric acid in an alkaline medium to form a reddish-yellow complex, the intensity of which is directly proportional to the concentration of creatinine in the specimen and can be measured at 520 nm (green filter).

Specimen: Serum (or heparinized plasma)

Q: What are the normal ranges (reference ranges) of serum creatinine?

Ans: Normal range: 0.7 to 1.7 mg/dl

Requirements

1. Test tubes: 15 × 125 mm
2. 5.0 ml serological pipettes
3. 1.0 ml and 2.0 ml volumetric pipettes
4. Test tube stand

5. Centrifuge tubes or test tubes, 100 × 10 mm
6. Centrifuge
7. Photometer

Preparation of the reagents

1. Picric acid reagent: 0.91 gm/dl (0.04 M)
2. 10 g/dl, sodium hydroxide
3. Working creatinine standards, 1 mg/dl, 5 mg/dl and 10 mg/dl

These standards are prepared in 0.01 N hydrochloric acid by using stock creatinine standard 100 mg/dl.

Stability of the reagents: Reagents 1 and 2 are stable at room temperature (25°C + 5°C). The working standards are stable at 2–8°C.

Preparation of alkaline picrate reagent: It is prepared fresh by mixing 4 parts of reagent 1 and 1 part of reagent 2. This working reagent is stable for one day.

Q: What is the SOP of the serum creatinine test?

Ans: Procedure: Pipettes in the tubes are labeled as:

Table 20.3

	Test	Std.
	(in centrifuge tubes)	
Distilled water, ml	3.0	4.0
Serum, ml	1.0	—
Standard 1 mg/dl, ml	—	1.0
2/3N sulfuric acid, ml	0.5	—
10 g/dl sodium tungstate ml	0.5	—

Centrifuge the contents in the test and get clear filtrate.

Pipettes in the tubes are labeled as follows:

Table 20.4

	Test	Std: 1	Blank
Distilled water. ml	3.0	3.0	5.0
The filtrate, ml	2.0	—	—
Diluted Std., 1 mg/dl, ml	—	2.0	—
Alkaline picrate reagent, ml	1.0	1.0	1.0

- Mix and keep at room temperature (25°C + 5°C) for 20 minutes.
- Read intensities of test and standard at 520 nm (green filter) by setting blank to 100% T.

Calculations

$$\text{Serum proteins, g/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 1.0$$

Procedure limitations

1. The method is not sensitive. In the case of the normal specimen, OD readings may fall below 0.1 to 0.050 OD.
2. Linearity of the method is up to 5.0 mg/dl. For sample values above 5.0 mg/dl, it is necessary to put up a higher standard, either 5.0 mg/dl or 10 mg/dl. The results are calculated by comparing the test readings with the higher standard reading.

Sources of error: The method is not accurate since other serum constituents (non-creatinine chromogens) like glucose, acetoacetate, and pyruvate also react with the alkaline picrate reagent and produce a similar colored complex.

Precautions: Saturated picric acid is poisonous and alkaline picrate reagent is corrosive. Contact with skin and clothing should be avoided.

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Determination of urine creatinine is necessary for creatinine clearance test.

Experiment 4: Determination of urine creatinine by alkaline picrate method

Clinical significance: Normal urinary excretion of creatinine is 1.5 to 3.0 g per 24 hours. The excretion rate decreases in all kinds of renal diseases and also in the post-renal conditions. The determination is of value in the calculation of creatinine clearance test results. (Refer to p496).

Requirements: All the requirements are the same as for serum creatinine determination.

Additional reagent: 3 g/dl, sulfosalicylic acid

Specimen: 24 hours urine specimen (a few thymol crystals are used as a preservative for the collected urine sample).

Procedure

a. Pipette about 5.0 ml of urine in a test tube.
b. Add 2–3 drops of 3 gm/dl, sulfosalicylic acid.

Observations

A. **Appearance of turbidity:** Urine contains proteins.
B. **No turbidity:** Urinary proteins absent.

A. If the urine contains proteins, deproteinize it by using the following method.

Table 20.5: Pipette in a centrifuge tube, the following requirements:	
a. Distilled water	8.0 ml
b. Urine	1.0 ml
c. 2/3 N sulfuric acid	0.5 ml
d. 10 g/dl sodium tungstate	0.5 ml
Mix well and centrifuge at 1,500 RPM for 10 minutes.	

B. If urine proteins are absent, dilute urine 1: 10 by using distilled water (9.0 ml of distilled water and 1.0 ml of urine) and mix thoroughly.

Now pipette in the tubes labeled as follows:

Table 20.6

	Test	Std	Blank
Distilled water, ml	4.8	4.8	5.0
Deproteinized, or diluted urine, ml	0.2	—	—
Standard 10 mg/dl, ml (undiluted)	—	0.2	—
Alkaline picrate reagent, ml	1.0	1.0	1.0

Mix, and keep at room temperature for 20 minutes. Take OD readings against blank at 520 nm (green filter).

Calculations

$$\text{Serum albumin g/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 100$$

For the determination of 24 hours creatinine excretion, measure the urine volume, and calculate the result as follows:

$$\text{Creatinine excretion, mg/24 hours} = \frac{\text{Urine creatinine, mg/dl ml quantity of 24 hours urine}}{100}$$

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.7: Demonstrate creatinine clearance

Determination of creatinine clearance

Introduction: Creatinine clearance test gives a relatively accurate and useful measure of the glomerular filtration rate and also the excretory capacity of the kidney. The reasons for the greater degree of accuracy of creatinine clearance are:

1. Creatinine is not absorbed by the tubules
2. The effect of fluid intake and excretion on creatinine clearance is much less than that of urea and,
3. The blood creatinine values are relatively stable. The creatinine clearance values may be greater than the actual glomerular filtration rate when plasma creatinine levels increase considerably above the normal range.

Clinical significance: Normal average creatinine clearance is 100 ml. Creatinine clearance values <75 ml indicate kidney dysfunction (renal disease). In severe renal disease, values of creatinine fall below 50 ml. Similarly, progress in the treatment of renal disease can be determined by the improved values of creatinine clearance.

Q. Define creatinine clearance.
Ans: The clearance of creatinine is defined as the number of ml of plasma that contains the amount of creatinine excreted in the urine in one minute.

Q: What is the formula used for the determination of creatinine clearance?

Ans: The following formula is used for the determination of creatinine clearance:

$$\text{Clearance} = \frac{UV}{S(P)} \times \frac{1.73}{A}$$

U = mg/ml of urine creatinine
S (P) = mg/ml of serum (or plasma) creatinine
V = ml of urine excreted per minute.
1.73 = Standard average surface area of a normal individual
A = Surface area of the patient

1.73/A is determined by referring to a standard chart in a textbook or a clinical laboratory. This is meant to correct clearance values according to body size since clearance varies according to body size.

Q: What are the normal values of creatinine clearance?

Ans: Normal values

a. For males: 105 + 20 ml/min.
b. For Females: 95 + 20 ml/min.

Preparation of the patient

Basic requirements:

1. A polythene container to collect 24-hour. urine. It should contain a few thymol crystals (preservatives).
2. Urine collection procedure:
   A. The patient should be instructed to empty the bladder at the beginning of the period (8 am) and discard the urine.
   B. Collect all urine passed until 8 o'clock the next morning, emptying the bladder at that time and adding this urine to the 24-hour specimen.
   C. The urine should be kept in a cool place.

NOTE

Diuretic drinks and drugs should not be given to the patient during the 24 hours of urine collection.

1. A blood sample is collected in a test tube for serum (preferably a fasting specimen).
2. Patient's height and age are noted for 1.73/A determination.

Laboratory determination requirements:

1. Patient's serum
2. 24-hour urine specimen
3. All other requirements are the same as those used for the determination of serum creatinine (Experiment No 3) and urinary creatinine (Experiment No 4).

Procedure

1. Measure the volume of the collected urine specimen.
2. Determine serum and urine creatinine.
3. Find out the ratio $\frac{1.73}{A}$ by referring to the standard chart.
4. Calculate creatinine clearance using the following formula:

Creatinine clearance, ml=

$$\frac{\text{Urine creatinine, mg/dl}}{\text{Serum creatinine, mg/dl}} \times \frac{V}{\text{min}} \times \frac{1.73}{A}$$

Experiment 5: Determination of uric acid by end point reaction—enzymatic method.

Q: What is the clinical significance of the serum uric acid test?

Ans: Clinical significance

Uric acid is the end product of nucleoprotein metabolism. It is a low-threshold excretory product. The serum uric acid level is often raised in gout. The determination has diagnostic value in differentiating gout from non-gouty arthritis. Uric acid levels are also increased in renal failure, uremia, and leukemia.

Q: What are the instructions given to the patient for the serum uric acid test?

Ans: Patient care, and preparation

The patient should be fasting for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material: Serum (or plasma)

Q: What method is used for serum uric acid test?

Ans: Uricase method

Q: What is the normal range of serum uric acid?

Ans: Normal range: 2.0–7.0 mg/dl

Q: What is the principle of serum uric acid (uricase method)?

Ans: Principle: Uricase in the reagent acts on uric acid and peroxidase in the reagent acts on hydrogen peroxide in the following reactions:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

Peroxidase present in the reagent acts on H_2O_2

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + (O)$$

The phenolic chromogens present in the reagent, 2, 4-dichlorophenol sulfonate (DCFS), and 4-aminophenazone get oxidized to form the red-colored compound, the intensity of which can be measured at 510 (500–530 nm, green filter). The concentration of the red-colored compound is proportional to the amount of uric acid in the specimen.

Reagents

1. Stock reagent in the lyophilized form contains the following components:
   - Buffer, pH 7.5: 100 mmol/L
   - Uricase
   - Peroxidase
   - Chromogen
2. Uric acid standard: 5.0 mg/dl.

Stability of reagents: The reagents are stable at 2–8°C

Preparation of working reagent: A working reagent is prepared by mixing the contents of the stock lyophilized reagent with distilled water. The working reagent is stable at 2–4°C for 60 days.

Q: What is the SOP of the serum uric acid test?

Ans: Procedure (Fig. 20.1)

Pipette in the tubes, labeled as follows:

Table 20.7

	Test	Std	Blank
Working reagent, ml	1.0	1.0	1.0
Serum, ml	0.02	–	–
Uric acid std, ml	–	0.02	–
Distilled water, ml	–	–	0.02
Mix well, keep the tubes at room temperature (25°C ± 5°C) for 10 minutes. Read absorbances of test and standard against blank at 510 nm (green filter).			

Calculations

Serum uric acid, mg/dl = OD Test/OD Std. × 5

NOTE

The method is linear up to 25 mg/dl

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.13: Demonstrate estimation of SGPT and SGOT.

Experiment 6A: Determination of serum glutamate pyruvate transaminase (SGPT) by end point reaction method.

Experiment 6B: Determination of glutamate oxaloacetate transaminase (SGOT) by end point reaction method.

Q: What is the clinical significance of SGPT and SGOT tests?

Ans: Clinical significance: Serum SGPT and SGOT levels are elevated in viral hepatitis and other forms of liver disease associated with hepatic necrosis. Levels of both these enzymes are elevated even before the clinical signs and symptoms of jaundice appear. Very high values (as high as 100 times the upper reference limit) of these enzymes are observed between the 7th and 12th days, with a subsequent decrease in activity. Normal levels of SGOT and SGPT reach by the 3rd or 5th week, if recovery is uneventful. Increased values of SGPT and SGOT may also be observed in extrahepatic cholestasis (post-hepatic conditions).

Five to tenfold elevations of both enzymes occur in a patient with primary or metastatic carcinoma, with AST usually being higher than ALT.

In drug-induced hepatitis, high values of ALT and AST are observed (similar to that in infectious hepatitis).

In alcoholic hepatitis, ALT and AST levels are moderately increased.

After myocardial infarction values of SGOT increase and reach peak values after 18 to

24 hours, and the activity values fall within the normal range by the fourth or fifth day (provided no new infarct has occurred).

Q: What are the instructions given to the patient for SGPT and SGOT tests?

Ans: Patient care and preparation: The patient should fast for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material: Serum

Q: What method is used for SGPT and SGOT tests?

Ans: Reitman and Frankel's end-point reaction method.

Q: What is the normal range of SGPT and SGOT?

Ans: Normal range

SGPT: 5–35 KU

SGOT: 8–40 KU

Q: Define Karmen units.

Ans: Enzyme units

Karmen units (KU): These are expressed as the unit of activity which produces a change in OD of 0.001 per minute by an enzyme present in 1.0 ml of serum.

Q: What is the principle of SGPT and SGOT tests?

Ans: Principle: Serum glutamate pyruvate transaminase (SGPT) catalyzes the following reaction:

$$\text{Alanine + alpha-ketoglutaric acid} \xrightleftharpoons{\text{SGPT}} \text{Pyruvic acid + glutamic acid}$$

Serum glutamate oxaloacetate transaminase (SGOT) catalyzes the following reaction:

$$\text{Aspartic acid + alpha-ketoglutaric acid} \xrightleftharpoons{\text{SGOT}} \text{Oxaloacetic acid + glutamic acid}$$

In the following procedure, the amount of pyruvic acid in the case of SGPT and oxaloacetic acid in the case of SGOT, are determined after incubation, colorimetrically by the formation of 'hydrazone' with dinitrophenyl hydrazine reagent (DNPH) which is highly colored, in alkaline medium (0.4 N sodium hydroxide).

Requirements

1. Test tubes: 15 × 125 mm
2. 5.0 ml serological pipettes
3. 0.1 ml serological pipettes
4. Constant temperature water-bath
5. Stopwatch
6. Photometer

Reagents

1. *SGPT substrate:* It contains alanine, and alpha-keto-glutaric acid, in phosphate buffer pH. 7.45 (M/15).
2. *SGOT substrate:* It contains aspartic acid, and alpha-ketoglutaric acid, in the phosphate buffer, pH, 7.45 (M/15).
3. *DNPH reagent:* It contains dinitrophenyl hydrazine and 8.5 % of conc. hydrochloric acid in distilled water; the final volume should be adjusted to one liter using distilled water.
4. 0.4 N sodium hydroxide
5. 22 mg/dl, sodium pyruvate standard.

Stability of the reagents: The reagents 1, 2, 3, and 5 are stable at 2–8°C. Reagent 4 is stable in a polyethylene container for several months at room temperature (25°C ± 5°C),

Sample material: Serum free from hemolysis, since, hemolysis interferes with the test. Use a fresh serum.

Q: What are the SOPs of SGPT and SGOT tests?

Ans: Procedure

Wavelength: 546 nm (green filter, 530–550 nm)

Incubation temperature: 37°C.

Incubation time:

— SGPT: 30 minutes

— SGOT: 60 minutes

NOTE

One blank is sufficient for each assay series. However, for high icteric sera, individual blanks are prepared. Readings are recorded in terms of OD against blank.

Procedure (SGPT determination): Pipettes in the tubes are labeled as follows:

Table 20.8

	Test	Blank
Substrate, ml	0.5	0.5
Incubate at 37°C for 5 minutes		
Serum, ml	0.1	—
Incubate at 37°C for 30 minutes		
DNPH, ml	0.5	0.5
Serum, ml	—	0.1
Mix thoroughly, keep at room temperature for (25°C + 5°C) 20 minutes.		
0.4 N NaOH, ml	5.0	5.0
Mix and keep at room temperature for 10 minutes. Afterward, read the intensity of the test by setting blank at 100% T (540 nm: green filter).		

Calculations

From the OD reading of SGPT find out corresponding KU by referring to the standard graph of SGPT.

Refer to standard graph (Fig. 20.3).

For "OD" readings of the "test", find out corresponding "enzyme units" from the graph.

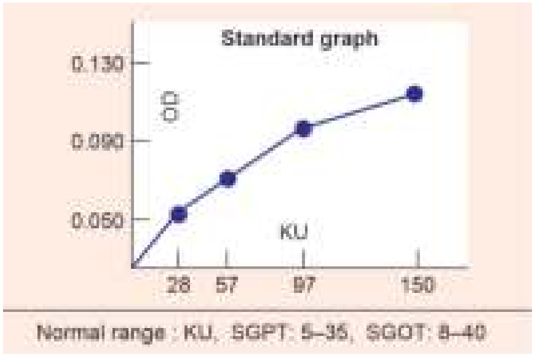


Fig. 20.3: A standard graph for SGPT

Procedure: (SGOT determination)

Pipettes in the tubes are labeled in Table 20.8:

Calculations

From the OD reading of SGOT find out corresponding KU by referring to the standard graph prepared for SGOT.

Refer to standard graph meant for SGOT, similar to Fig. 20.3.

Procedure limitations: For values, more than 150 for SGPT and 190 for SGOT, dilute serum 1: 10 and repeat the assay (Result × 10).

Preparation of a standard graph for SGPT determination

Table 20.9

	Test	Blank
Substrate, ml	0.5	0.5
Incubate at 37°C for 5 minutes		
Serum, ml	0.1	—
Incubate at 37°C for 60 minutes (1 hour)		
DNPH, ml	0.5	0.5
Serum, ml	—	0.1
Mix thoroughly, and keep at room temperature for (25°C + 5°C) for 20 minutes.		
0.4 N NaOH, ml	5	5
Mix and keep at room temperature for 10 minutes. Afterward, read the intensity of the test by setting blank at 100% T (540 nm, Green filter).		

Pipettes in the tubes are labeled in Table 20.10:

Table 20.10

	1	2	3	4	Blank
SGPT substrate, ml	0.45	0.4	0.35	0.3	0.5
Std, sodium pyruvate, ml	0.05	0.1	0.15	0.2	—
Distilled water, ml	0.1	0.1	0.1	0.1	0.1
DNPH, ml	0.5	0.5	0.5	0.5	0.5
Mix. thoroughly, and keep at room temperature (25°C ± 5°C) for 20 minutes.					
0.4 NaOH, ml	5	5	5	5	5
Karmen Units	28	57	97	150	0
Mix and keep at room temperature for 10 minutes. Read intensities by setting blank at 100% T at 546 nm (green filter).					

Draw a curve by plotting OD on Y-axis and Karmen units on X-axis (Fig. 20.1).

Preparation of a standard graph for SGOT determination.

Pipettes in the tubes are labeled in Table 20.11:

Table 20.11

	1	2	3	4	Blank
SGOT substrate, ml	0.45	0.4	0.35	0.3	0.5
Std. sodium pyruvate, ml	0.05	0.1	0.15	0.2	—
Distilled water, ml	0.1	0.1	0.1	0.1	0.1
DNPH, ml	0.5	0.5	0.5	0.5	0.5
Mix thoroughly, and keep at room temperature (25°C ± 5°C) for 20 minutes.					
0.4 NaOH, ml	5	5	5	5	5
Karmen Units	27	61	114	190	0
Mix and keep at room temperature for 10 minutes. Read intetnsities by setting blank at 100% T at 540 nm (green filter).					

Draw a curve by plotting OD on Y-axis and Karmen units on X-axis, similar to Fig. 20.3.

Precautions: Reagent 4 is highly corrosive; handle it with care.

Interfering substances: Drugs and toxic substances which are detoxified by the liver cells can result in elevated values of SGOT and SGPT. Other drugs which also elevate these enzymes are paracetamol overdose, chlorpromazine, methyl testosterone, and steroids in contraceptive pills.

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.14: Demonstrate estimation of serum alkaline phosphatase

Experiment 7: Determination of serum alkaline phosphatase (S. ALP) by end point reaction method.

Q: What is the clinical significance of the alkaline phosphatase test?

Ans: Clinical significance: Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions:

a. Hepatobiliary disease and
b. Bone disease associated with increased osteoblastic activity.

Moderately elevated levels are observed in hepatic conditions, and the elevation tends to be more marked in post-hepatic conditions. Among the bone diseases, the highest levels of serum ALP activity are encountered in Paget's disease. Only moderate rises are observed in osteomalacia. In rickets, levels two to four times the normal are observed, which drop to normal values after treatment with vitamin D. Very high levels of S. ALP are found in bone cancer, and slight to moderate elevations of the enzyme are found in hyperparathyroidism.

Q: What are the instructions given to the patient for the serum alkaline phosphatase test?

Ans: Patient care and preparation:

The patient should be fasting for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material: Serum

Q: What is the name of the method for the serum alkaline phosphatase test?

Ans: Name of the method

P-nitrophenyl phosphate (PNP) method.

Q: What is the principle of the serum alkaline phosphatase test?

Ans: Principle

Para-nitrophenyl phosphate is colorless. The enzyme splits off the phosphate group from it to form para-nitrophenol, which in the acid form in dilute solution is also colorless. Under alkaline conditions, this is converted to P-nitro phenoxide ions, which exhibit yellow color. The intensity of the yellow color is directly proportional to the enzyme present in the specimen and can be measured at 405 nm. (violet filter).

Q: What is the normal range of serum alkaline phosphatase test?

Ans: Normal range

- 20–90 IU (adults).
- 93–221 IU (children).

Requirements

1. Test tubes (15 × 125 mm)
2. 5.0 and 0.2 ml serological pipettes
3. Constant temperature water bath
4. Photometer

Preparation of reagent

1. AMP buffer (pH 10.3)
2. P-nitrophenyl phosphate powder (PNP)
3. 30 mg/dl magnesium chloride reagent
4. 0.25 N sodium hydroxide
5. P-nitrophenol standard: 30 µm/dl (4.173 mg/dl)

Stability of the reagents: Reagents 1, 3, 5, and PNP powder are stable at 2–8°C. Reagent 4 is stable in a polyethylene container at room temperature (25°C + 5°C).

Procedure

- Wavelength: 405 nm (violet filter).
- Incubation temperature: 37°C.
- Incubation time: 15 minutes.

NOTE

One blank is sufficient for each assay series. For high icteric sera, however, individual blanks are prepared.

Preparation of working substrate: Prepare fresh by dissolving 42.5 mg of P-nitrophenyl phosphate in 0.5 ml of magnesium chloride solution (sufficient only for one test). This buffered substrate is stable at 2–8°C for one day.

Q: What is the SOP for the serum alkaline phosphatase test?

Ans: Procedure

Pipette in the tubes labeled as follows:

Table 20.12

	Test	Blank
AMP, buffer, ml	2.7	2.7
Working substrate, ml	0.2	0.2
Mix, and keep at 37°C for 5 min.		
Serum, ml	0.1	–
Mix, keep at 37°C for 15 minutes		
Serum, ml	–	0.1
0.25 N NaOH, ml	3.0	3.0

Mix, keep at room temperature (25° + 5°C) for 5 minutes. Read intensity of test against blank at 405 nm (violet filter).

Calculations

From the OD reading of serum alkaline phosphatase (SAP) find out corresponding IU by referring to the standard graph of SAP

Refer to standard graph (Fig. 20.4).

The results are expressed in terms of **international units (IU)** and can be expressed as micromoles of product formed per minute per liter of the specimen.

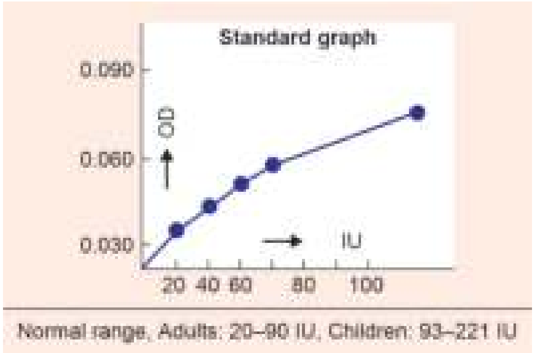


Fig. 20.4: Standard graph for serum alkaline phosphatase

Preparation of a standard graph

Pipette in the tubes are labeled as follows:

Table 20.13

	Blank	1	2	3	4
AMP, Buffer, ml	2.8	2.7	2.6	2.4	2.0
Working nitrophenol, ml	0.0	0.1	0.2	0.4	0.8
Magnesium chloride ml	0.2	0.2	0.2	0.2	0.2
Mix thoroughly, keep at room temperature (25°C + 5°C) for 5 minutes.					
0.25 NaOH, ml	3.0	3.0	3.0	3.0	3.0
Mix thoroughly, keep at room temperature for 5 minutes.					
Read against blank at 405 nm (Violet filter).					
I.U.	0	20	40	80	160

Prepare a graph by plotting IU on X-axis and OD reading on Y-axis. (Fig. 20.4).

Procedure limitations

1. The method may not obey Beer's law.
2. For sample values above 160 IU, dilute the sample and repeat the assay (Result × 10).

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.12: Demonstrate estimation of serum bilirubin

Experiment 8: Serum total, direct and indirect bilirubin.

Q: What is the clinical significance of the serum bilirubin test?

Ans: Clinical significance: Serum bilirubin increases pre-hepatic, hepatic, and post-hepatic jaundice.

Pre-hepatic conditions are caused by the excessive formation of indirect bilirubin due to hemolytic anemia. Very high concentrations of indirect bilirubin may cause intra-hepatic cholestasis leading to disturbances in the bile pigment metabolism, leading to jaundice.

Hepatic conditions are caused mainly due to hepatitis A and hepatitis B viruses and also by hepatitis C, hepatitis D, hepatitis E and hepatitis G viruses. SARS-CoV-2 and parasites such as malarial parasites and amoebae also infect liver cells. Bacteria such as leptospira also infect the liver. Various drugs such as paracetamol, sulphonamides, anti-tuberculosis drugs, and alcohol also may cause drug-induced hepatitis.

Post-hepatic conditions are caused due to extra-hepatic cholestasis, caused by gall stones in the bile duct, bile duct carcinoma, or due to bile duct stricture.

Q: What are the instructions given to the patient for the serum bilirubin test?

Ans: Patient care and preparation: The patient should be fasting for at least 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material: Serum

NOTE

A morning blood sample (preferably for serum) from a fasting subject is preferred to avoid lipemia. Hemolysis of serum should be avoided since it produces falsely low values with diazo methods.

Both conjugated and unconjugated bilirubin are photo-oxidized when exposed to white or UV light. Specimens should be protected from direct exposure to either artificial light or sunlight as soon as they are drawn.

Storage of specimens in the dark and at low temperatures is essential (2°–8°C) in the refrigerator.

Q: What is the name of the method for the serum bilirubin test?

Ans: Method: Malloy and Evelyn.

Q: What is the principle of serum bilirubin test?

Ans: Principle: The method is based on the van den Bergh reaction. When bilirubin reacts with diazo reagent, purple-colored azobilirubin is formed. Methanol is used as a reaction accelerator. Since total bilirubin (indirect and direct type) is soluble in it. By using only distilled water, direct bilirubin is determined. The difference between total bilirubin and direct bilirubin gives a measure of indirect bilirubin. The optical densities of the total test and direct test are measured against respective blanks at 540 nm (green filter, 510–560 nm).

Q: What is the normal range of serum bilirubin

Ans: Normal range

- Total bilirubin up to 1.0 mg/dl
- Direct bilirubin up to 0.5 mg/dl
- Indirect bilirubin up to 0.5 mg/dl

Requirements

1. Test tubes: 15 × 125 mm
2. 5.0, 0.2 ml serological pipettes
3. Stopwatch
4. Test tube stand
5. Photometer

Reagents

1. Diazo 'A'
2. Diazo 'B'.
3. Diazo blank reagent
4. Methanol
5. 10 mg/dl artificial bilirubin standard

Stability of the reagents: Reagents 1, 3, 4 and 5 are stable at room temperature (25°C + 5°C) for one year. Reagent 2 is stable at 2–8°C in an amber-colored bottle.

Q: What is the SOP of serum bilirubin test?

Ans: Test procedure: Prepare a fresh diazo mixture by mixing 5.0 ml of Diazo A and 0.15 ml of Diazo B. This mixture is stable only for a day.

Pipettes in the tubes are labeled as follows:

Table 20.14

	Total	Direct	Direct	
	Test	Blank	Test	Blank
Distilled water, ml	1.8	1.8	1.8	1.8
Serum, ml	0.2	0.2	0.2	0.2
Diazo mixture, ml	0.5	—	0.5	—
Diazo blank reagent, ml	—	0.5	—	0.5
Methanol, ml	2.5	2.5	—	—
Distilled water, ml	—	—	2.5	2.5

Keep in the dark for 30 minutes. Read the intensities at 540 nm (green filter).

Read the OD of the artificial bilirubin standard (undiluted) by transferring the standard solution in a dry cuvette at 540 nm (or Green filter).

Calculations

OD of total bilirubin = OD of total test – OD of total blank

OD of direct bilirubin = OD of direct test – OD of direct blank.

$$\text{Total bilirubin mg/dl} = \frac{\text{OD of total bilirubin}}{\text{OD of std.}} \times 100$$

$$\text{Direct bilirubin mg/dl} = \frac{\text{OD of total bilirubin}}{\text{OD of std.}} \times 100$$

Indirect bilirubin, mg/dl = Total bilirubin, mg/dl – Direct bilirubin, mg/dl.

Sources of error

1. Hemolysis inhibits the diazo reaction.
2. Exposure to light decreases bilirubin in the sample.

Procedure limitations: The method is linear up to 20.0 mg/dl. For sample values above 20 mg/dl, dilute the sample and repeat the test. Apply proper dilution factor for the calculation of the final result.

Quality control: To ensure adequate quality control, the use of commercial reference serum is recommended.

Additional information: The disadvantage of this simple and otherwise very satisfactory method is that owing to the dilutions used (1:25), not much color is developed when the serum bilirubin is low (i.e. about 1.0 mg/dl). If more serum is used to overcome this, some turbidity results from slight precipitation of proteins.

LIPID PROFILE TESTS

Enumerate lipid profile tests

1. Serum total cholesterol
2. Serum high-density lipoprotein (HDL)/ cholesterol

3. Total cholesterol/HDL cholesterol ratio
4. Serum triglycerides
5. Low-density lipoprotein (LDL)
6. Very low-density lipoprotein (VLDL)

Q: What is the clinical significance of lipid profile tests?

Ans: Clinical significance

1. Elevation of the total cholesterol values in plasma is considered to be a prime risk factor for coronary heart disease.
2. Increased triglycerides and VLDL values are taken as primary risk factors. A low serum triglyceride level is suggestive of intravascular lipolysis and enhanced formation of HDL. Hypertriglyceridemia, on the other hand, indicates less effective intravascular lipolysis and a reduced formation of HDL, which is associated with a higher atherogenic risk.
3. Elevated LDL is suggestive of atherogenic risk. A low level of HDL cholesterol indicates a high risk of coronary heart disease.
4. Total cholesterol/HDL-cholesterol ratio should be below 5.0. If this ratio is more than 5.0, then it is usually regarded as a risk factor in the development of ischemic heart disease.

Q: What are the pre-analytical considerations for lipid profile tests?

Ans: Pre-analytical considerations

Reliable determination of lipids and lipoproteins is dependent on the control of both analytical and pre-analytical factors. The pre–analytical aspects include biological variations, behavior factors, clinical factors, and variability in sample collection and handling.

The following are the recommendations of the National Cholesterol Education Program's (NCEP) Laboratory Standardization Panel (LSP), USA:

- The individuals should be on their usual diet.
- Their weight should be stable for at least 2 weeks before the determination of serum lipids.
- Multiple determinations within 2 months (at least 1 week apart) should be made before a medical decision is made for further action.
- Patients should not engage in vigorous physical activity within 24 hours before the lipid panel tests.
- For all lipid panel tests (except serum total cholesterol) a 12 hours fasting sample is required.
- Patients should be seated for 5 minutes before collection of the sample.
- The tourniquet should be released within 1 minute during venipuncture.
- Serum or plasma (heparinized) should be removed from cells within 3 hours of venipuncture.
- Specimen can be stored at 4°C for up to 3 days and up to several weeks at –20°C.
- Serum or plasma can be stored and transported either at 4°–0°C or frozen.

Competency achievement: The student should be able to:

BI11.9: Demonstrate estimation of serum cholesterol and HDL-cholesterol

Experiment 9: Determination of serum total cholesterol by enzymatic method

Q: What is the clinical significance of serum total cholesterol test?

Ans: Clinical significance: Elevated levels of serum cholesterol are associated with atherosclerosis, nephrosis, diabetes mellitus, obstructive jaundice and myxedema. Decreased levels are observed in hyperthyroidism, malabsorption and anemia.

Sample material

Q: What are the instructions given to the patient for serum total cholesterol test?

Ans: Patient care and preparation

The patient should be fasting for at least 8–12 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material: Serum

Serum (fasting): Hemolysed serum interferes with the test

Q: What method is used for the determination of total cholesterol?

Ans: Enzymatic method is used for the determination of total cholesterol?

Q: What is the principle of the test?

Ans: Principle: Cholesterol esters present in the serum are hydrolyzed by cholesterol ester hydrolase to free cholesterol and fatty acids. The free cholesterol produced and pre-existing ones are oxidized by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Peroxidase acts on hydrogen peroxide and liberated oxygen reacts with the chromogen (4-amino phenazone/phenol) to form a red-colored compound which is read at 510 nm (505–530 nm)

Reagents

1. Buffer/enzymes/chromogen: It contains cholesterol ester hydrolase cholesterol oxidase and peroxidase dissolved in 100 ml of phosphate buffer (M/10, pH 7.0) containing 4-aminophenazone.
2. Phenol reagent
3. Cholesterol standard: 200 mg/dl.

Stability of the reagents: All the reagents are stable at 2–8°C.

Preparation of working reagent: It is prepared fresh by mixing two parts of reagent 1 and one part of reagent 2. This reagent is stable for 12 hours at 2–8°C in an amber-colored container.

Q: What is the SOP of the total cholesterol test?

Ans: Procedure (Fig. 20.1)

Pipettes in the tubes are labeled as follows:

Table 20.15

		Test	Std.	Blank
1.	Working reagent, ml	3.0	3.0	3.0
2.	Serum, ml	0.02	—	—
3.	Standard, ml	—	0.02	—
4.	Distilled water, ml	—	—	0.02

Mix well and keep at 37°C for 20 minutes. Read absorbance of test and standard against blank.

Calculations

$$\begin{array}{c}\text{Serum total}\\ \text{cholesterol, mg/dl}\end{array} = \frac{\text{OD test}}{\text{OD std.}} \times 200$$

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Procedure limitations: The method is linear up to 500 mg/dl. For sample values above 500 mg/dl, dilute the specimen 1:10 and repeat the test (result × 10).

NOTE

The enzymatic methods are subject to interference from other colored substances (bilirubin, hemoglobin, etc.) and those compounds (ascorbic acid) that compete with oxidation reactions.

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.10: Demonstrate estimation of serum triglycerides

Experiment 10: Determination of serum triglycerides by enzymatic method.

Q: What is the clinical significance of the serum triglycerides test?

Ans: Clinical significance: Elevated levels of serum triglycerides are associated with an increased risk of atherosclerosis, heart attack, stroke and pancreatitis.

Sample material

Q: What are the instructions given to the patient for serum triglycerides test?

Ans: Patient care, and preparation: The patient should be fasting for at least about 12 hours.

Sample material

Serum (fasting): Hemolysed serum interferes with the test.

Q: What method is used for the determination of serum triglycerides?

Ans: Enzymatic method is used for the determination of serum triglycerides?

Q: What is the normal range of serum triglycerides?

Ans: Normal range of serum triglycerides: 10–190 mg/dl

Q: What is the principle of the test?

Ans: Principle of test: Lipoprotein lipase in the reagent acts on serum triglycerides:

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{fatty acids}$$

Glycerol kinase in the reagent acts on glycerol:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

Glycerol phosphate oxidase in the reagent acts on glycerol-3-phosphate

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phos-phate oxidase}} \text{Dihydroxy acetone phosphate} + H_2O_2$$

Peroxidase the reagent acts on hydrogen peroxide:

$$H_2O_2 + \text{4-amino-phenazone} + \text{p-chlorophenol} \xrightarrow{\text{Peroxidase}}$$

The formation of a colored complex takes place. It is measured at 520 nm (green filter).

Reagents

1. Buffer/enzymes/chromogen: It contains –
    a. Lipoprotein-lipase
    b. Glycerol kinase
    c. Glycerol phosphate oxidase
    d. Peroxidase
    e. Glycerol phosphate in phosphate buffer, pH: 7.2 (50 mmol/L).
2. p-Chlorophenol reagent
3. Triglyceride standard: 100 mg/dl

Preparation of working reagent: It is prepared fresh by mixing two parts of reagent 1 and one part of reagent 2.

Stability: Reagents 1 and 2 are stable at 2–8°C.

Q: What is the SOP of serum triglycerides test?

Ans: Procedure (Fig. 20.1)

Pipettes in the tubes are labeled as follows:

Table 20.16

		Test	Std.	Blank
1.	Working reagent, ml	3.0	3.0	3.0
2.	Serum, ml	0.02	–	–
3.	Standard, ml	–	0.02	–
4.	Distilled water, ml	–	–	0.02

Mix well, and keep at 37°C for 15 minutes. Read absorbance of test and standard against blank.

Calculations

$$\text{Serum triglycerides, mg/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 100$$

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Q: What is the method for the determination of HDL-cholesterol?

Ans: Method: Immuno-inhibition

Determination of HDL-cholesterol by immunoinhibition method

This method is based on inhibition and can be performed manually as well on autoanalyzers.

Requirements

1. **Reagent 1:** This reagent contains an antibody to human apo B-100 that reacts with the apo B-100 containing lipoproteins such as chylomicrons, VLDL, and LDL. This reaction blocks the reaction of cholesterol contents of chylomicrons, VLDL, and LDL
2. **Enzymes reagent containing cholesterol esterase, cholesterol oxidase, and**

peroxidase: Enzymes present in this reagent act on esterified and free cholesterol of HDL fraction as shown in the enzymatic method of cholesterol determination, and the OD of color produced at the end of the reaction is directly proportional to the concentration of serum HDL-cholesterol.

Q: What method is used for the determination of serum VLDL and LDL-cholesterol?

Ans: Determination of serum VLDL and LDL-cholesterol

Indirect method: Fairly accurate determination of VLDL and LDL can be done for the values of cholesterol less than 400 mg/dl, by using the following formulae based on "The Friedewald equation".

1. Determine:
   a. Total cholesterol
   b. HDL-cholesterol and c. TG.
   Then calculate VLDL and LDL as follows:
   a. VLDL mg/dl = Triglyceride/5
   b. LDL-cholesterol, mg/dl = Cholesterol (total) – HDL-cholesterol – Triglyceride/5

Competency achievement: The student should be able to:

BI11.11: Demonstrate estimation of serum calcium and inorganic phosphorus

Experiment 11: Determination of serum (or plasma) calcium by CPC method.

Q: What is the clinical significance of the serum calcium test?

Ans: Clinical significance: Decreased serum calcium values are found in hypoparathyroidism, rickets, osteomalacia and steatorrhea. A decrease in serum calcium can occur in acute pancreatitis and in those forms of renal diseases in which excessive proteinuria is observed. Increased serum calcium values are observed in hyperparathyroidism, hypervitaminosis D and multiple myeloma.

Sample material

Q: What are the instructions given to the patient for the serum calcium test?

Ans: Patient care, and preparation: The patient should be fasting for at least about 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material

Serum (fasting): Hemolysed serum interferes with the test

Q: What method is used for the determination of serum calcium?

Ans: Cresolphthalein complexion

Q: What is the normal range of serum calcium?

Ans: 8.5–9.5 mg/dl

Q: What is the principle of the test

Ans: Principle: Calcium reacts directly with cresol phthalein complexion (CPC) reagent containing dimethyl sulfoxide and 8-hydroxyquinoline. Since magnesium also reacts with CPC, the addition of 8-hydroxyquinoline virtually eliminates the interference from magnesium.

Requirements

1. Test tube (15 × 125 mm)
2. 100 ml graduated cylinder
3. 100 ml beaker
4. 10 ml graduated pipette
5. Push-button pipette (0.05 ml)
6. Stopwatch
7. Photometer

Reagents

1. **Calcium reagent 1:** Cresolpthalein complexion containing 8-hydroxyquinoline.
2. **Calcium reagent 2:** Diethylamine containing potassium cyanide
3. **Calcium standard:** 10 mg/dl (5.0 mEq/L): It contains 25 mg of Calcium carbonate in 50% (v/v) hydrochloric acid.
4. EDTA: 4.0 g/dl

Stability of the reagents: Reagents 1 and 2 are stable at room temperature for 3 months. Reagent 3 is stable at 2–8°C, and reagent 4 is stable at room temperature for several months.

NOTE

It is necessary to use glass distilled water (prepared from only a glass distillation plant)

Q: What is the SOP for the serum calcium test?

Ans: Procedure

Prepare fresh working reagent by mixing equal quantities of reagent 1 and reagent 2 (X ml of reagent 1 and X ml. of reagent 2).

NOTE

The color of the working reagent should be light purple. This working reagent is stable only for a day at room temperature (25° + 5°C).

Pipettes in the tubes are labeled as follows:

Table 20.17

	Test	Std.	Blank
Working reagent, ml	6.0	6.0	6.0
Serum or (heparinized) plasma, ml	0.05	–	–
Standard 10 mg/dl, ml	–	0.05	–
Distilled water, ml	–	–	0.05

Mix thoroughly and keep at room temperature for exactly 10 minutes. Read intensities of test and standard against blank at 575 nm (yellow filter).

Calculations

$$\text{Urine creatinine, mg/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 100$$

Precautions

1. Keep all glassware scrupulously clean and use only chromosulfuric acid for cleaning.
2. For blood collection and the determination of calcium, use dry glassware, finally rinsed with distilled water.
3. Use disposable tips.

Procedure limitations: If the calcium concentration exceeds 15 mg/dl, dilute the sample appropriately and apply the dilution factor for the final calculations.

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Experiment 12: Determination of serum inorganic phosphorus by direct UV-determination without reduction.

Q: What is the clinical significance of the serum inorganic phosphorus test?

Ans: Clinical significance: Decreased serum phosphorus values are observed in preliminary hyperparathyroidism, rickets, and Fanconi's syndrome (a disease associated with a defect in the reabsorption of phosphorus). Increased serum phosphorus levels may be found in hypervitaminosis D, hypoparathyroidism, and renal failure.

Sample Material

Q: What are the instructions given to the patient for the serum inorganic phosphorus test?

Ans: Patient care preparation: The patient should be fasting for at least about 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material

Serum (fasting): Hemolysed serum interferes with the test

Q: What are the normal values of serum inorganic phosphorus?

Ans: Normal values

Adults	2.5–4.5 mg/dl
Children	4.0–7.0 mg/dl

Q: What is the name of the test?

Ans: Direct UV determination.

Q: What is the principle of inorganic phosphorus test?

Ans: Principle: Inorganic phosphorus present in the specimen reacts with ammonium molybdate in the presence of sulfuric acid to form phosphomolybdate which is measured at 340 nm. The change in OD is directly proportional to the amount of inorganic phosphorus. It is compared with a known standard.

Reagents

1. **Phosphorus reagent:** It contains ammonium molybdate, sulfuric acid, sodium chloride, and Tween 20 (surfactant)
2. Inorganic phosphorus standard: 5 mg/dl

Q: What is the SOP for the serum calcium test?

Ans: Procedure: Pipettes in the tubes are labeled as follows:

Table 20.18

		Test	Std.	Blank
a.	Phosphorus reagent, ml	1.0	1.0	1.0
b.	Serum, ml	0.02	–	–
c.	5 mg/dl, standard, ml	–	0.02	–
d.	Distilled water, ml	–	–	0.02

Mix well and after 2 minutes, read the absorbance of the test and standard against blank at 340 nm.

Calculations

$$\text{Serum phosphorus, mg/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 5$$

Quality control: The use of commercial reference control sera is recommended to ensure adequate quality control.

Competency achievement: The student should be able to:

BI11.8: Demonstrate estimation of serum proteins, albumin, globulins, and A/G ratio

Experiment 13: Determination of total serum protein by Biuret method.

Q: What is the clinical significance of serum total proteins determination?

Ans: Clinical significance: An increase in total proteins may occur in dehydration. Both albumin and globulin are increased due to hemoconcentration.

A decrease in total proteins is always due to a low albumin level, accompanied either by no increase in globulin or an increase in globulin so the ratio of A/G is changed. Low serum albumin may be due to the following:

1. A heavy loss of albumin in the urine (as in nephritis).
2. Malabsorption of amino acids from the alimentary tract (as in steatorrhea).
3. Decreased formation in the liver (as in cirrhosis of the liver).
4. Increased catabolism of proteins (as in fever) or
5. Insufficient intake of proteins in the food (malnutrition)

An increase in globulin occurs in:

1. Advanced liver disease,
2. Multiple myeloma
3. In the number of chronic infections such as tuberculosis, rheumatoid arthritis, sub-acute bacterial endocarditis, lupus erythematosus disseminates, etc.

Q: What is edema?

A reduction in the total proteins is one of the causes of edema. It may take place when total proteins fall below about 5.0 g/dl and albumin below about 2.5 g/dl. In this condition, water from blood passes into tissue spaces. Plasma proteins may also be decreased in acute or chronic hemorrhage.

Sample material

Q: What are the instructions given to the patient for serum total proteins and albumin tests?

Ans: Patient care and preparation: The patient should be fasting for at least about 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material

Serum (fasting): Hemolysed serum interferes with the test.

Q: What is the normal range of serum total proteins?

Ans: Normal range

Serum proteins: 6–8 g/dl

Q: What method is used for the determination of serum total proteins?

Ans: Name of the method: Biuret method

Q: What is the principle of the Biuret method?

Ams: Test principle: Proteins react with cupric ions in an alkaline medium to form a violet-colored complex. The intensity of the color produced is directly proportional to proteins present in the specimen and can be measured on a photometer at 530 nm (or by using a green filter).

Requirements

1. Test tubes: 15 × 125 mm
2. Serological pipettes, 5 ml
3. Test tube stand
4. Push button pipette of 0.05 ml or serological pipette of 0.1 ml
5. Photometer

Reagents:

1. Working protein reagent (working Biuret reagent—ready to use).
2. Protein standard: 6.0 g/dl: 6 g of bovine albumin dissolved in 100 ml of normal saline, containing 0.1 g/dl, sodium azide.

Additional reagents

Sample blank reagent

Stability of the Reagents

Reagents 1 and 3 are stable at room temperature (25° + 5°C) for one year. Reagent 3 (protein standard) is stable at 2–8°C for one year.

Q: What is the SOP of Biuret test?

Ans: Procedure

Pipette in three tubes labeled as follows:

Table 20.19

	Test	Std.	Blank
Protein reagent, ml	5.0	5.0	5.0
Serum, ml	0.05	–	–
Protein std. 6 g/dl, ml	–	0.05	–
Distilled water, ml	–	–	0.05

Mix thoroughly and keep at room temperature (25°C + 5°C) for exactly 10 minutes. Measure the intensities of the test and standard by setting blank at 100% T, by using 530 nm (green filter).

Calculations

$$\text{Serum proteins, g/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 6$$

Sources of error: Icteric (containing high bilirubin) and Lipemia sera may interfere with the test.

Quality control: To ensure adequate quality control for both methods, the use of commercial reference control serum is recommended.

Experiment 14: Determination of serum albumin by BCG method.

Q: What is the clinical significance of the serum albumin test?

Ans: A decrease in total proteins is always due to a low albumin level, accompanied either by no increase in globulin or an increase in globulin so the ratio of A/G is changed. Refer to clinical significance of Experiment 13.

Sample material

Q: What are the instructions given to the patient for serum total proteins and albumin tests?

Patient care and preparation: The patient should be fasting for at least about 8 hours. A fasting specimen is preferred, however, any random blood specimen also can be used.

Sample material

Serum (fasting): Hemolysed serum interferes with the test.

Q: What is the normal range of serum albumin?

Ans: Normal range: 3.3–4.8 g/dl.

Q: What method is used for the determination of serum albumin?

Ans: Name of the method

Bromocresol green (BCG) method.

Q: What is the principle of the BCG method?

Ans: Principle: Albumin present in serum binds specifically with bromocresol green at pH 4.1 to form green colored complex, the intensity of which can be measured colorimetrically by using 640 nm (or a red filter).

Requirements

1. Test tubes: 15 × 125 mm
2. Serological and graduated pipettes, 10 ml, 5 ml
3. Test tube stand
4. Push-button pipette of 0.05 ml or serological pipette of 0.1 ml
5. Photometer
6. Serum

Reagents

1. Albumin reagent (ready to use): It contains Bromocresol green, pH 4.1
2. Albumin standard, 4.0 g/dl: 4.0 g Bovine albumin is dissolved in 100 liters of normal saline containing 0.1 g/dl sodium azide.
3. Sample blank reagent

Stability of the reagents

- Reagents 1 and 3 are stable at room temperature (25°C + 5°C) for one year.
- Reagent: 2 (albumin standard) is stable at 2–8°C for one year.

Q: What is the SOP of the BCG test?

Ans: Procedure

Pipettes in the tubes are labeled as follows:

Table 20.20

	Test	Std.	Blank
Albumin reagent, ml	5.0	5.0	5.0
Serum, ml	0.05	–	–
Albumin standard, ml	–	0.05	–
Distilled water, ml	–	–	0.05

Mix thoroughly and keep at room temperature (25° + 5°C) for exactly 10 minutes. Measure the intensity of the test and standard by setting blank at 100% T, by using 640 nm (red filter).

Calculations

$$\text{Serum albumin, g/dl} = \frac{\text{OD test}}{\text{OD std.}} \times 4$$

Source of error: Icteric (containing high bilirubin) and lipemia sera may interfere with the test.

Calculations

$$\frac{\text{OD test}}{\text{OD std.}} \times 4$$

Procedure limitations: The linearity of this method is up to 6.0 g/dl.

Quality control: To ensure adequate quality control for both methods, the use of commercial reference control serum is recommended.

Q: How to determine total globulins and A/G ratio?

Ans: Determination of globulins

Serum globulins, g/dl = Total proteins, g/dl - Albumin, g/dl.

Normal range = 1.8–3.6 g/dl

$$\text{A/G ratio} = \frac{\text{Serum albumin, g/dl}}{\text{Serum globulin, g/dl}}$$

Q: What is the normal range of A/G ratio?

Ans: Normal range of A/G ratio:

1.2:1 to 2:1

Competency achievement: The student should be able to:

BI11.15: Describe and discuss the normal composition of CSF

Q: Describe and discuss the normal composition of CSF.

Ans: The cerebrospinal fluid (CSF) is formed by selective dialysis of plasma by the choroid plexus of the ventricles of the brain. Through the foramina in the fourth ventricle, it then passes into subarachnoidal cisterns at the base of the brain and travels over the surfaces of the cerebral hemispheres. It is finally absorbed into the blood in the cerebral veins and dural sinuses. CSF is present in the cavity that surrounds the brain in the skull and the spinal cord in the spinal column. The volume of CSF (adults) is about 150 ml. CSF performs the following functions.

1. It helps to protect the brain and spinal cord from injury by acting like a fluid buffer.
2. It also acts as a medium for the transfer of substances from the brain tissue and spinal cord to blood.
3. It maintains intracranial pressure.

Normal composition of CSF

1. Color: Colorless
2. pH: 7.3–7.4
3. Appearance: Clear
4. Clot formation: No clot formation on standing
5. Specific gravity:1.003–1.008
6. Total solids: 0.85–1.70 g/dl
    - Protein : 15 to 45 mg/dl (Albumin = 50–70%, Globulins = 30–50%)
    - Glucose : 40–80 mg/dl
    - Chlorides : 700–750 mg/dl
    - Sodium : 144–154 mEq/L
    - Potassium : 2.0–3.5 mEq/L
    - Creatinine : 0.5–1.2 mg/dl
    - Cholesterol : 0.2–0.6 mg/dl
    - Urea : 6–16 g/dl
    - Uric acid : 0.5–4.5 mg/dl
7. Cells: 0–8 lymphocytes/per cumm (µl), Neutrophils: Absent

Competency achievement: The student should be able to:

BI11.3: Describe the chemical composition of normal urine

Q: What is the normal composition of urine?

Ans: Composition of normal urine

1. Volume: 600–2000 ml/24 hours. Average: 1,200 ml.
2. Specific gravity: 1.003–1.030
3. Reaction: Acidic (pH: 4.7–7.5). Average pH: 6.0
4. Total solids: 30–70 g/liter.

Q: What urine constituents are excreted per 24 hours?

Ans: The following are the normal constituents of urine that are excreted per 24 hours:

Table: 20.21: Urinary constituents excreted in 24 hours

	Constituent	Quantity excreted/24 hours
1.	Urea	25–30 g
2.	Creatinine	1–1.8 g
3.	Uric acid	0.3–1.0 g
4.	Creatine	60–150 mg
5.	Hippuric acid	0.1–1.0 g
6.	Sodium	3–4 g
7.	Potassium	1.5–2.0 g
8.	Chlorides	9–16 g
9.	Calcium	0.1–0.3 g
10.	Inorganic phosphorus	1–1.5 g
11.	Sulfur	0.7–3.5 g
12.	Magnesium	0.05–0.2 g
13.	Ammonia	0.3–1.0 g
14.	Iodine	50–250 µg
15.	Purine bases	7–10 mg
16.	Ketone bodies	3–15 mg
17.	Coproporphyrins	60–280 µg
18.	Vitamins, hormones, and enzymes:	Detected in small quantities

ROUTINE URINE EXAMINATION

Q: What type of specimen is used for routine urine examination?

Ans: Type of specimen

First voided midstream morning urine.

NOTE

1. The concentration of urine varies throughout 24 hour period. It depends on the water intake of a person and partly on his activities. A random urine specimen collected during the daytime may be diluted and may not be suitable for the detection of certain substances present in low concentrations. Hence more concentrated urine is preferred for testing, which can be obtained by collecting first voided morning urine.

2. For urgent, routine examination, however, to get a general idea of the expected pathological condition, a random urine specimen may be used.

Q: What type of container is used for routine urine examination?

Ans: The container used for urine collection:

Clean and dry wide-mouth glass or plastic bottles, with screwcap tops. (capacity, about 250–300 ml). The bottles need not be sterile.

Q: What instructions are given to the patient for the collection of urine for routine urine examination?

Ans: Instructions given to the patient:

The patient should be instructed to void directly into the container. During the collection, the initial portion of the urine stream is allowed to escape while the midstream portion is collected.

Q: How urine specimens from infants and young children are collected?

Ans: Specimens from infants and young children can be collected in a disposable collection apparatus. It consists of a plastic bag with an adhesive backing about the opening to fasten it to the child so that the patient voids directly into the bag. Care must be taken to avoid fecal contamination.

NOTE

1. All the specimens for routine urinalysis should be examined while fresh (within one hour of the collection).
2. When urine is kept for longer than one hour before analysis, to avoid deterioration of chemical and cellular material and to prevent the multiplication of bacteria, it should be stored at 2–8°C in a refrigerator.

Q: How urine is collected for 24 hours and its purpose?

Ans: For the qualitative tests, the morning urine is useful, however, quantitative tests are performed only on urine specimens collected for 24 hours.

24-hour urine collection: All urine specimens from 8 AM to the next day 8 AM are collected in a plastic container, containing 2–3 thymol crystals as preservative.

For an adult, the normal average daily volume of urine is about 1200–1500 ml.

DEMONSTRATE PHYSICAL EXAMINATION OF URINE (AS THE PART OF ROUTINE EXAMINATION OF URINE)

Experiment 15: Physical examination of urine using multi-stixs

Specimen: Freshly collected first-morning sample.

Other requirements:

1. Pasteur pipettes
2. Ordinary filter papers
3. Measuring cylinder
4. Litmus papers and pH papers (range 2–10.5)
5. Urinometer (Fig. 20.5): This is a bulb-shaped instrument that has a cylindrical stem that contains a scale calibrated in specific gravity readings. This instrument is floated in a cylinder containing urine. The depth to which it sinks in the urine indicates the specific gravity of urine, which is read on the urinometer scale at the junction of the urine with the air.

Procedure

1. Observe the following aspects of the urine specimen and note them down in the notebook: (a) Color, (b) Appearance, (c) Odor, and (d) Sediment (if present).
2. Determination of urine volume: Measure the volume of urine and note it down in the notebook.
3. Determination of urine reaction and pH
   - Place a drop of urine by using a Pasteur pipette (or a glass rod) on a blue litmus paper and note down the reaction.

     Change of blue litmus to red:
     Reaction: Acidic. No change in color:
     Reaction: Alkaline.

- Place a drop of urine on a pH paper (range 2–10.5)), and from the color change, note the pH value.

4. Determination of urine specific gravity

   Procedure:

   a. Mix urine well and fill the container three-fourths full of urine.
   b. Remove all foam by using filter paper.
   c. Float the urinometer in the urine. Rotate it carefully so that it can be prevented from touching the bottom or sides of the container.
   d. Note the specific gravity reading from the scale.

   Urinometer (sp. gr. meter) must float centrally in the urine specimen, without touching the sides of the cylinder (or container).

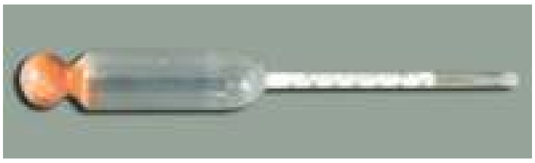


Fig. 20.5: Urinometer (sp. gr. meter)

Q: What is the clinical significance of a physical urine examination?

Ans: The various aspects studied for the physical examination of urine are:

1. Volume
2. Color
3. Appearance
4. Sediment formation
5. Odor
6. Reaction and pH
7. Specific gravity (sp.gr.).

Clinical significance

1. Volume

The normal quantity of first voided urine is about 50–250 ml.

Urine volume of more than 500 ml is observed in diabetes mellitus and diabetes insipidus.

Oliguria means excretion of urine less than 400 ml per day. Anuria means complete suppression of urine formation despite normal or high fluid intake. In renal and post-renal diseases oliguria is observed.

2. Color

Normal color: Pale yellow

In hepatic and post-hepatic jaundice color of urine is dark yellow.

The presence of blood also gives reddish-brown color to urine.

Many factors and constituents can alter the normal urine color. These include diet, medicines, and various chemicals that can be present in disease.

3. Appearance:

Normal urine is usually clear.

It may appear cloudy if amorphous phosphates are present in alkaline urine or amorphous urates are present in acidic urine.

Urine may appear cloudy or turbid from the presence of leukocytes and epithelial cells. The presence of red blood cells may give urine a turbid and smoky appearance.

4. Sediment formation:

If the urine contains amorphous phosphates, amorphous urates, a large number of leukocytes, epithelial cells, etc., on standing it for some time, sediment formation will take place at the bottom of the container.

5. Odor:

The normal odor of urine: Usual, distinct normal odor.

The presence of ketone bodies gives urine a sweet or fruity smell. Contaminated urine with bacteria may give a pungent smell due to the formation of ammonia. The urine of an infant with phenylketonuria gives a musty odor.

6. pH:

Use a pH paper to determine the pH of urine Freshly collected urine's normal pH is acidic. The alkaline pH of fresh urine may be observed in bacterial infection of the kidneys.

7. Specific gravity

Normal range for a random specimen is 1.003 to 1.035.

Usually, the specific gravity rises when the fluid intake is low, and decreases when the fluid intake is high.

In diabetes insipidus, the specific gravity of urine is very low, while in diabetes mellitus, the specific gravity is high due to the presence of glucose in urine.

The high specific gravity of urine is observed in various conditions such as dehydration, eclampsia, proteinuria, lipoid nephrosis, etc.

DEMONSTRATE CHEMICAL EXAMINATION OF URINE USING MULTI-STIX

CHEMICAL EXAMINATION OF URINE BY USING MULTI-STIX REAGENT STRIPS (Fig. 20.6)

Q: What are multi-stix reagent strips? Explain the uses of multi-stix strips.

Ans: Multi-stix reagent strips are clear plastic strips. Seven different reagent areas are affixed on the strip. These different cellulose areas are impregnated with specific testing chemicals according to the test.

The various determinations possible by multi-stix reagent strip are:

1. pH
2. Protein
3. Glucose
4. Ketones
5. Bilirubin
6. Occult blood, and
7. Urobilinogen.

The reagent strips are also available for only one or two tests, such as

a. Glucose
b. Glucose and protein
c. Glucose and ketones, or
d. Bilirubin and urobilinogen, etc.

In addition, test strips are also available for the detection of phenyl pyruvic acid, bacteria, pus cells, mucopolysaccharides, etc.

Q: What are the advantages of multi-stix reagent strips?

Ans: The advantages of chemical examination of urine by multi-stix strips are as follows:

- It gives quick screening of urine chemistry.
- The method is very fast (requires only 2 to 3 minutes).
- The method is reliable, specific, and sensitive.
- It avoids the use of various corrosive reagents, different types of glassware, and other laboratory materials required for wet chemical testing of urine.
- It can be performed on uncentrifuged urine and does not require acidification. (Centrifuged urine is preferred for wet chemical examination of urinary proteins, which may require further acidification.)

Experiment 16: Chemical examination of urine using multi-stix reagent strips.

Requirements

1. Uncentrifuged urine
2. Multi-stix reagent strips

Procedure (Fig. 20.6)

1. Dip the test areas of the strip in the urine specimen (fresh, well mixed, and uncentrifuged).
2. Remove excess urine by tapping the edge of the strip against the container.
3. Compare the test areas closely with corresponding color charts on the bottle at the times specified.
4. Note the findings in a notebook.

NOTE

The strip should be compared with the corresponding color chart on the strip bottle, in reasonably good light (Fig. 20.6).

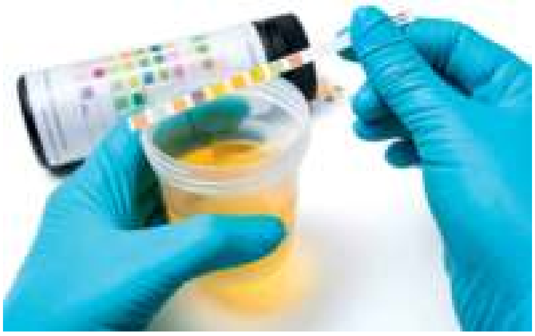


Fig. 20.6: Chemical examination of urine by multi-sticks

Test	Color change
pH	The test area changes from orange to yellow and green through blue.
Protein	The test area changes from a shade of green to blue in the presence of proteins.
Glucose	The test area changes from sky-blue to green to chocolate brown.
Ketones	The test area changes from pale grey to form a violet dye complex.
Bilirubin	The test area changes from buff through various shades of tan or tannish-purple.
Urobilinogen	The test area changes from brown to orange.
Occult blood	The test area changes from orange to green to dark blue.

Q: What principles are involved in the chemical examination of urine by multi-sticks?

1. **Determination of pH**

   Principle: The indicators used in the test area are methyl red (pH range: 4.4–6.2, color change: Red to yellow) and bromothymol blue (pH range: 8.0–9.6, color change: Yellow to blue). When the test strip is dipped in urine, the color of **the reaction area changes from orange to yellow and green to blue**. It depends on the pH of the urine, e.g. if the color is yellow, the pH may be about 6.0, and if the color is blue, the pH may be about 9.0. The test area is compared with the corresponding color charts on the bottle (Fig. 20.6).

2. **Determination of proteins**

   Principle: The test area is impregnated with tetra bromophenol blue buffered to an acid pH. This area is yellow in the absence of protein, but at the same pH, **changes to a shade of green to blue in the presence of proteins.** The change in the test area depends upon the concentration of proteins present.

3. **Determination of glucose**

   Principle: The test area is impregnated with glucose oxidase and peroxidase enzymes and the chromogen KI. If glucose is present in urine, glucose oxidase acts on it, and the following reactions take place.

   $$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + \text{hydrogen peroxide}$$

   $$\text{Hydrogen peroxide} \xrightarrow{\text{Peroxidase}} \text{water} + \text{oxygen}$$

   Oxygen reacts with the chromogen (KI), and the original color of the **test area (sky blue) changes from green to chocolate brown,** depending upon the concentration of glucose in urine. The test is specific only for glucose.

4 **Determination of ketones**

   Principle: The reaction area contains sodium nitroprusside, glycine, and a buffer. The sodium nitroprusside reacts with diacetic acid and acetone in an alkaline medium **to form a violet dye complex.** The test is more sensitive to acetoacetic acid than to acetone. It does not react with β-hydroxybutyric acid.

5. **Determination of bilirubin**

   Principle: The reaction area contains either 2–4 dichloroaniline diazonium salt. If bilirubin is present in urine, it will couple with the diazonium salts mentioned above. The color changes from **buff to various shades of tan or tannish-purple.**

6. **Determination of urobilinogen**

   Principle: The reaction area contains p-dimethylamino benzaldehyde, which reacts with urobilinogen in a strongly acidic medium to produce a **color change from brown to orange.**

7. **Determination of occult blood**

   Principle: The procedure is based on the peroxidase-like activity of hemoglobin, which catalyzes the oxidation of an indicator such as tetramethylbenzidine by an organic peroxidase. **The color change is from orange to green to dark blue.**

DEMONSTRATE MICROSCOPIC EXAMINATION OF URINE

Experiment 17: Microscopic examination of urine

General consideration: The microscopic examination is a valuable diagnostic tool for the detection and evaluation of renal and urinary tract disorders and other systemic diseases.

Normal urine sediment examinations show very few pus cells, epithelial cells, and a few crystals.

Principle: The microscopic elements present in urine (in suspension) are collected in the form of deposits by centrifugation. A small drop of the sediment is examined by making a coverslip preparation under a microscope.

Requirements

1. Centrifuge tubes or test tubes (10 × 75 mm).
2. Glass slides
3. Coverslips
4. Pasteur pipettes

Instruments

1. Centrifuge
2. Microscope

Specimen

Freshly voided, midstream, morning urine

Procedure

1. Mix the urine and pour it into a centrifuge tube (or small test tube) until it is 3/4 full (about 5 ml).
2. Centrifuge with another balanced test tube for 5 minutes at 2,500 RPM.
3. Pour off the supernatant quickly and completely into another test tube (this can be used for protein determination).
4. Resuspend the deposit by shaking the tube.
5. Place one drop of the deposit on a glass slide.
6. Cover it with a coverslip and mark it with the identification number.
7. Observe it first under low power objective in subdued light. This is obtained by partially closing the iris diaphragm and then adjusting the condenser downward until satisfactory contrast is obtained. Note the contents of various fields.

NOTE

1. The fine adjustment should be continuously adjusted up and down. It enables the viewer to see objects and other structures which may be on different focal planes.
2. Switch to a high dry objective and observe at least 10 to 15 different fields.

Observations

1. Normal urine sediment shows: Occasional pus cells, epithelial cells, and a few crystals
2. In urinary tract infections, the following types of components are observed in large numbers: Pus cells, epithelial cells, casts sometimes crystals (if calculi are present) (Figs 20.7 to 20.11).

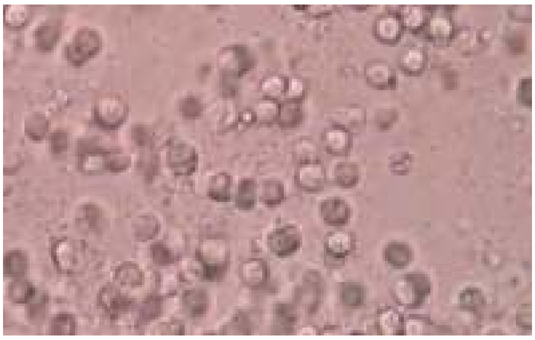


Fig. 20.7: Pus cells

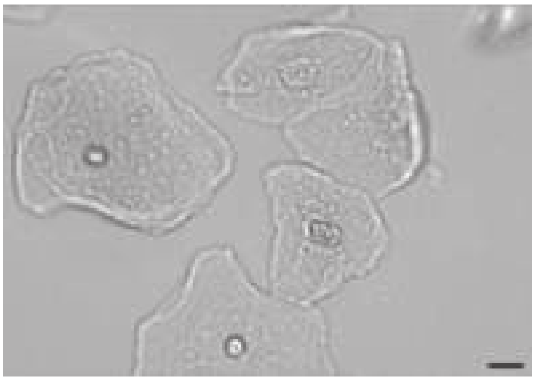


Fig. 20.8: Squamous epithelial cells

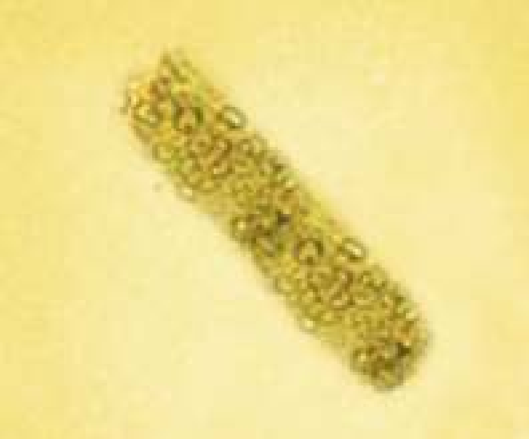


Fig. 20.9: Granular cast

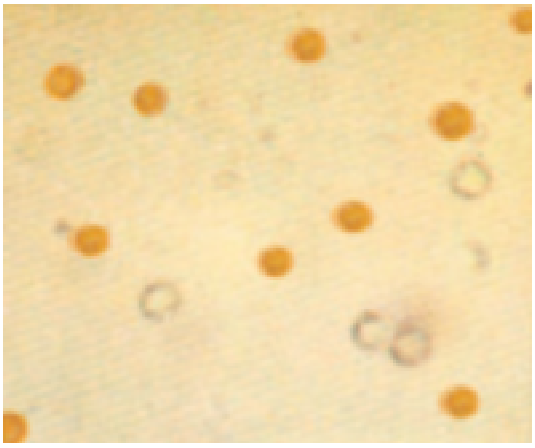


Fig. 20.10: Calcium oxalate crystals

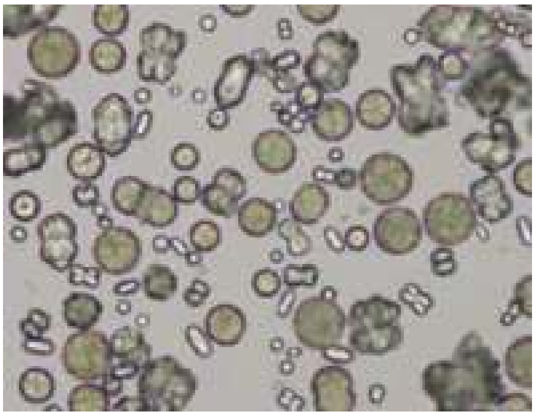


Fig. 20.11: Uric acid crystals

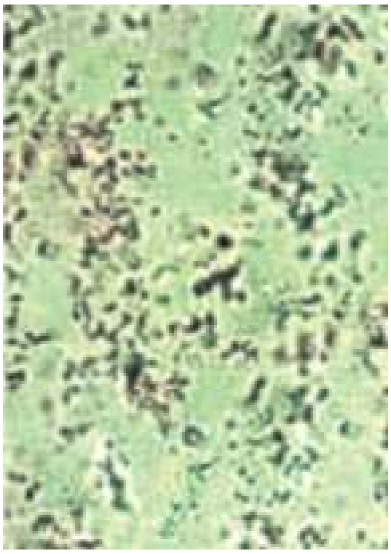


Fig. 20.12: Amorphous crystals

Competency achievement: The student should be able to:

BI11.16: Observe working of a biochemistry autoanalyzer

WORKING OF A BIOCHEMISTRY AUTOANALYZER

Example: Plasma glucose test:

1. Perform plasma glucose test (Fig. 20.1, Experiment 1)
2. Put on the semi-autoanalyzer (Fig. 20.13)

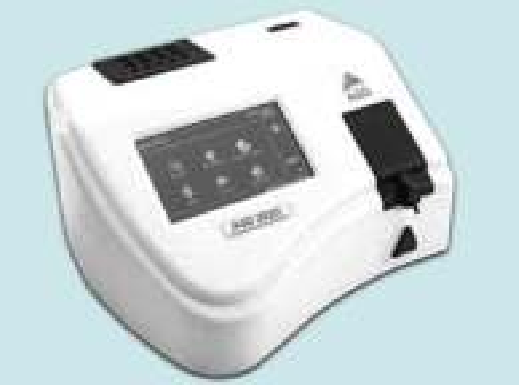


Fig. 20.13: Biochemistry semi-autoanalyzer

3. Introduce Blank (Fig. 20.14)

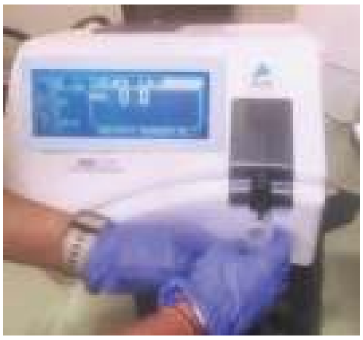


Fig. 20.14: Introduction of Blank: Reading on the screen is 00

4. Introduce "standard" 100 mg/dl (Fig. 20.15). The standard OD reading is displayed on the screen with the factor (100/OD of STD) for the calculation of test (serum/plasma) glucose.

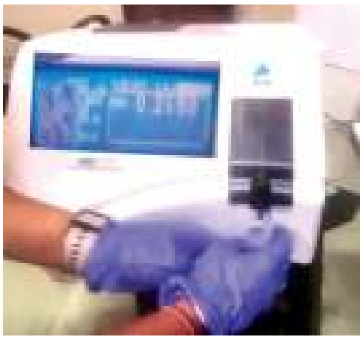


Fig. 20.15: Introduction of standard. Factor 0.2650 is displayed on the screen.

5. Introduce "test" (Fig. 20.16). Serum (or plasma) glucose value is displayed on the screen of the analyzer.

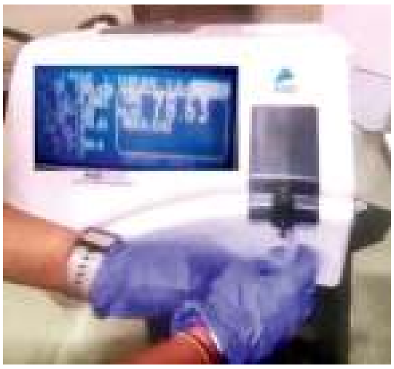


Fig. 20.16: Display of serum (or plasma) glucose: 78.63 mg/dl on the screen of the analyzer

OBSERVE SERUM AMYLASE TEST

Experiment 18: Determination of serum amylase by colorimetric (amyloclastic)

Clinical significance: Determination of serum and urine amylase is largely used in the diagnosis of diseases of the pancreas and the investigation of pancreatic function. In acute pancreatitis, a transient rise in serum amylase activity occurs within 2 to 12 hours of the onset. Serum amylase levels return to normal by the third or fourth day. Usually, high levels of amylase (four to sixfold elevations above the reference limit) are observed during 12 to 72 hours. The parallel increase in serum lipase confirms acute pancreatitis. The elevation of serum amylase activity is reflected in the rise of urinary amylase activity. The urinary clearance of amylase is markedly increased in acute pancreatitis.

Amylase assays are also important in detecting the development complications, such as pseudocyst, pleural effusion and ascites following acute pancreatitis. Serum amylase also increases in traumatic lesions of the pancreas (including surgical trauma and radiological investigations) and carcinoma of the pancreas.

Tumors of the lungs and serous tumors of the ovary can produce high levels of serum amylase with elevations as high as 50 times the upper reference limit. Both kinds of tumors can produce pleural effusion.

Method

Colorimetric: Amyloclastic, lodometric

Principle: Amylase in the specimen acts on the substrate, starch. The products formed are dextrins and maltose. After the incubation, when the end products are treated with the color reagent (iodine reagent), a decrease in the blue color is observed, compared to that produced with the blank. The disappearance of the blue color is directly proportional to the amylase concentration in the specimen and gives the measure of amylase present in the specimen.

Enzyme units

Caraways units (CU): Caraway defined the enzyme unit as the amount of enzyme that will hydrolyze 10 mg of starch in 30 minutes to a colorless stage. (In the procedure, the substrate should be completely hydrolyzed by 800 units of amylase in 100 ml serum.)

Normal range

60–180 caraway units

Sample material

Serum: The enzyme is quite stable. Activity loss is negligible even at room temperature. It is stable for at least 3 months when refrigerated.

Requirements

1. Test tubes: 15 × 125 mm
2. 5.0 ml, 10.0 ml and 0.1 ml serological pipettes
3. Constant temperature water-bath
4. Stopwatch
5. Photometer

Preparation of the reagents

1. **Buffered substrate:** It is prepared by mixing 2.66 anhydrous disodium hydrogen phosphate, 0.86 g of benzoic acid and 0.04 g of starch in 100 ml of distilled water.
2. **Stock color reagent:** It is prepared by mixing 0.3567 g of potassium iodate. 4.5 g of potassium iodide and 0.9 ml of conc. hydrochloric acid in 100 ml of distilled water.

Working color reagent

Dilute stock color reagent 1:10 in distilled water to get the working color reagent. It should be prepared fresh for the test.

Stability of the reagents

1. Reagent 1 is stable at 2–8°C for 3 months.
2. Reagent 2 is stable at 2–8°C for one year.

Procedure

Pipettes in the tubes are labeled as follows:

Table 20.22

		Test	Blank
1.	Buffered substrate, ml Keep at 37°C for 5 minutes	2.5	2.5
2.	Serum, ml Mix, and incubate at 37°C for 7½ min.	0.1	—
3.	Working color reagent, ml	2.5	2.5
4.	Serum, ml	—	0.1
5.	Distilled water, ml	20.0	20.0

Mix thoroughly, and read the intensities of the test and blank against distilled water at 660 nm (red filter).

Calculations

Serum amylase, Caraway units:

$$\frac{\text{OD blank} - \text{OD test}}{\text{OD blank}} \times 400$$

Procedure limitations: For the results above 400 Caraway units (CU), dilute serum 1:5 in distilled water and repeat the test, for calculations, use the appropriate dilution factor (Result × 5).

QUALITATIVE EXPERIMENTS

Experiment 19: Identification of a carbohydrate solution.

Test

Molisch test

Principle of the test

Molisch reagent reacts with carbohydrate solutions in the presence of concentrated sulfuric acid to form purple color. Carbohydrates are dehydrated by the action of sulfuric acid to form furfuryl derivatives. Non-carbohydrates do not react with Molisch reagent in the presence of sulfuric acid and furfuryl derivatives are not formed.

Requirements

1. Test tubes (20 × 150 mm)
2. 5.0 ml, 10.0 ml graduated pipettes
3. Pasture pipettes
4. Molisch qualitative reagent

Test Solutions

1. 250 mg/dl glucose
2. 250 mg/dl fructose
3. 250 mg/ dl sucrose
4. 100 mg/dl starch
5. 60 mg/dl protein solution
6. 100 mg/dl palmitic acid.

Test solutions 1, 2, 3 and 4 are prepared in distilled water. For the preparation of starch solution, hot water is used. Protein solution is prepared by mixing 1 ml of serum (6.0 g/ dl protein) in 99 ml of distilled water. Palmitic acid is prepared by dissolving 100 mg palmitic acid in 100 ml ethanol.

Molisch Qualitative Reagent

It is prepared by dissolving 2 g of α-naphthol in 20 ml of 96% ethyl alcohol.

Procedure

1. Pipette 1.0 ml of test solutions in appropriately labeled test tubes.
2. Add 3 drops of Molisch reagent to each test.
3. Add approximately 1.0 ml sulfuric acid to each test solution using Pasteur pipette slowly from the side of the test tubes, without mixing.

Results

All the tubes containing only carbohydrates (tubes 1, 2, 3 and 4) show purple color at the interphase between sulfuric acid and test solutions. Other test solutions, which contain non-carbohydrates do not show purple color.

NOTE

The reagents are highly corrosive, use hand gloves and safety glasses.

Experiment 20: Determination of urine glucose by Benedict test (Fig. 20.17).

Test

Benedict's qualitative: It is prepared as follows:

Place 173 g sodium citrate and 100 g sodium carbonate in a two liters beaker. Add one liter distilled water, mix and boil till solution becomes clear. Cool to room temperature (25° ± 5°C). Add 17.3 g cupric sulphate, and mix till it dissolves completely. Make final volume to one liter using a measuring cylinder of one liter. Store at room temperature (25° ± 50°C).

Principle of the Test

When Benedict's qualitative reagent (5 ml) is heated with eight drops of urine (about 0.5 ml), glucose present in urine reduces cupric ions present in the reagent to cuprous ions. Alkaline medium is provided to the reaction by sodium carbonate present in the reagent. The original color of Benedict's reagent is blue. It changes to green, yellow, orange or red, according to the concentration of glucose present in urine (Fig. 20.17).

NOTE

The test is nonspecific for glucose since the reaction may be brought by other carbohydrates such as fructose, galactose, lactose, and pentoses and also by noncarbohydrates such as ascorbic acid, salicylates, creatine and uric acid.

Requirements

1. Test tubes (20 × 150 mm)
2. 5.0 ml, 10.0 ml graduated pipettes
3. 500 ml beaker
4. Pasteur pipettes
5. Benedict's qualitative reagent
6. Bunsen burner

Procedure

1. Pipette 5.0 ml of Benedict's reagent in a test tube (20 × 150 mm).
2. By using Pasteur pipette, add eight drops (0.5 ml) of urine.
3. Heat carefully on the flame of a gas burner (or spirit lamp) or place in a boiling water (in a 500 ml beaker) for 5–10 minutes.
4. Cool under tap water or by placing in a beaker containing tap water.

Table 20.23: Observations

Color	Conclusion glucose mg/dl	Approximate
1. Blue	Sugar: Absent	Nil
2. Green and slight yellow precipitate	Sugar: Present, trace	250–500
3. Green and thick yellow precipitate	Sugar: Present, + to + +	500–750 750–1000
4. Yellow and orange precipitate	Sugar: Present, + + +	1000–1500
5. Orange and orange	Sugar: Present, ++++	More than 1500 to red ppt.

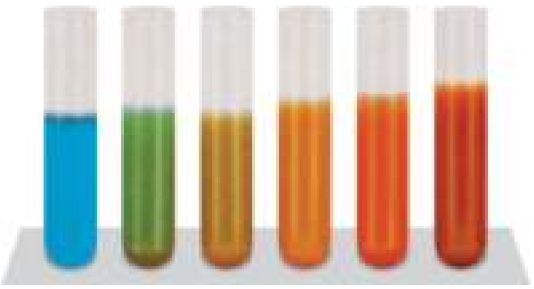


Fig. 20.17: Benedict test results

Note

If Benedict's test is positive then it is necessary to confirm presence of glucose by using glucose-oxidase uristix (Fig. 20.5). If glucose is present by uristix method, then use the above mentioned tabular observations to grade the results.

If glucose is absent then it may be necessary to identify the reducing substance present in urine. The substances of clinical interest are: (1) Lactose (2) Galactose and (3) Fructose. These are tested as follows:

- Lactose (for lactosemia)
- Fructose (for fructosemia)
- Galactose (for galactosemia)

The tests are performed in the following order:

- Osazone test (to identify lactose)
- Selivanoff's test (to identify fructose)
- Orthotoluidine test (in the absence of glucose, to identify galactose).

Experiment 21: Selivanoff's test (detection of lactose)

Test

Osazone test

Requirements

1. Test tubes (20 × 150 mm)
2. 5.0 ml, 10.0 ml graduated pipettes
3. 500 ml beaker
4. Pasteur pipettes
5. Glacial acetic acid
6. Sodium acetate
7. Phenylhydrazine hydrochloride
8. Litmus papers
9. Bunsen burner
10. Microscope

Principle

Monosaccharides and the two disaccharides lactose and maltose react with phenylhydrazine hydrochloride in acidic medium and after placing in boiling water bath, to form characteristic phenylhydrazone crystals. Lactose gives lactosazone crystals which are specific and can be identified by microscopic observation. These crystals look different from the osazone crystals formed by monosaccharides and maltose (Fig. 4.4).

Procedure

1. Transfer about 5 ml of sugar solutions respectively to the test tubes (20 × 150 mm).
2. Make it just acidic to litmus paper by adding a few drops of glacial acetic acid.
3. Add about one gram of the powder mixture of sodium acetate and phenylhydrazine hydrochloride (2 parts to 1).
4. Place in a boiling water bath for 30 minutes.
5. Cool by placing in a beaker containing tap water.
6. Observe collected deposit under microscope by making coverslip preparations.

OBSERVATIONS

Refer **Fig. 4.4** to identify, the crystals and related sugars.

Experiment 22: Orthotoluidine test (for detection of galactose)

Test

Orthotoluidine test (it is performed only if lactose and glucose are absent in the test solution, since they also react with orthotoluidine).

Reagent

Orthotoluidine reagent: It is prepared by dissolving 1.5 g thiourea in 940 ml glacial acetic acid and 60 ml of orthotoluidine.

NOTE

This reagent is highly acidic, hence should not be pipetted by mouth.

Principle

Orthotoluidine reagent reacts with galactose in hot acidic medium to form a green colored compound.

Requirements

1. Test tubes (20 × 150 mm)
2. 0.1 ml graduated pipettes
3. 5.0 ml push-button pipette (or a burette)
4. 500 ml beaker
5. Orthotoluidine reagent
6. Bunsen burner

Procedure

1. Pipette 5 ml of orthotoluidine reagent using a push-button pipette (or a burette) in a test tube (20 × 150 mm)
2. Add 0.05 ml of test sugar solution
3. Place in a boiling water bath for 10 minutes.
4. Observe the color.

Observations

1. No green color (original color of the reagent persists, i.e. pale yellow color): Galactose absent.
2. Green color (if lactose and glucose are absent): Galactose present.

Experiment 23: Selivanoff's test for the detection of fructose.

Test

Selivanoff's test

Reagent

Selivanoff's reagent: It is prepared by dissolving 50 mg of resorcinol in 33 ml of concentrated hydrochloric acid and diluted to 100 ml with distilled water.

NOTE

This reagent is highly acidic, hence should not be pipetted by mouth.

Principle

Hydrochloric acid acts on fructose to form a derivative of furfuraldehyde which gives red colored compound when linked with resorcinol.

Requirements

1. Test tubes (20 × 150 mm)
2. 5.0 ml push button pipette (or a burette) and 1.0 ml graduated pipettes
3. 500 ml beaker
4. Pasteur pipettes
5. Selivanoff reagent
6. Bunsen burner

Procedure

1. Pipette 5 ml of Selivanoff's reagent in a test (20 × 150 mm) using a push button pipette or a burette.
2. Add 0.5 ml of test sugar solution.
3. Heat to boil (or place in a boiling water bath for minutes).
4. Observe the color.

Observations

1. No change in color: Fructose absent
2. Color changes to red: Fructose present

Experiment 24: Observation of presence of protein in a solution.

Test

Protein: Qualitative test

Principle

Protein in a solution is not visible. If 2–3 drops of 3% (W/V) sulphosalicylic acid is added to the test solution, it becomes turbid due to denaturation of protein in the test solution.

Requirements

1. Test tubes: 15 × 125 mm
2. Pasteur pipette
3. Test solution: It is prepared by mixing 2.0 ml of any serum sample in 98 ml of normal saline.

Reagents

1. 3 g/dl, sulfosalicylic acid: It is prepared by mixing 3.0 g of sulphosalicylic acid in about 90 ml distilled water and final quantity is adjusted to 100 ml using distilled water.
2. Normal saline: It is prepared by mixing 0.85 g/dl sodium chloride in about 90 ml distilled water and final quantity is adjusted to 100 ml using distilled water.

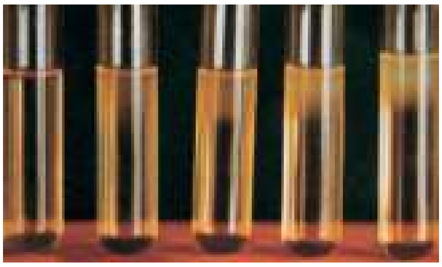


Fig. 20.18: Protein qualitative test results: First tube at LHS does not contain protein (No turbidity at the top of the tube is observed). Other tubes show presence of protein in variable quantities

Procedure

1. Transfer 5.0 ml of test solution in a test tube.
2. Add 2–3 drops of sulphosalicylic acid using a Pasteur pipette and mix well.
3. Observe the appearance of the test solution.

Observations

Appearance of test solution changed from clear to turbid due to denaturation of protein. This test indicates that the test solution contains proteins.

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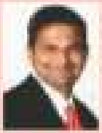

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